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APPLICATION NUMBER: ***60/177,823***
FILING DATE: ***January 25, 2000***

PRIORITY DOCUMENT

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01/25/00 U.S. PTO

Express Mail No.: EJ 676 402 031 US
Docket Number: 5953L1-01CA

jc511 U.S. PTO 60/177023 01/25/00

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et Number: 5953L1-01CA

PROVISIONAL APPLICATION FOR PATENT COVER SHEET (Large Entity)

This is a request f r filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)/APPLICANT(S)

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☐ *Additional inventors are being named on page 2 attached hereto*

TITLE OF THE INVENTION (280 characters max)

CALCIUM DICABOXYLATE ETHERS, METHODS OF MAKING SAME, AND TREATMENT OF VASCULAR DISEASE AND DIABETES THEREWITH

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification	*Number of Pages* 56		
☐ Drawing(s)	*Number of Sheets*	☒ Other (specify)	45 Claims on 11 Pgs., Abstract on 1 Pg

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

		FILING FEE AMOUNT
☐ A check or money order is enclosed to cover the filing fees		
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: ______

Respectfully submitted,

SIGNATURE Charles W. Ashbrook DATE January 25, 2000

TYPED or PRINTED NAME Charles W. Ashbrook REGISTRATION NO. *(if appropriate)* 27,610

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

SEND TO: *Box Provisional Application, Assistant Commissioner for Patents, Washington, DC 20231*

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INVENTOR(S)/APPLICANT(S)		
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5953L1-01CA

CALCIUM DICABOXYLATE ETHERS, METHODS OF MAKING SAME, AND TREATMENT OF VASCULAR DISEASE AND DIABETES THEREWITH

DM_FILE/PD 5953L1

CALCIUM DICABOXYLATE ETHERS, METHODS OF MAKING SAME, AND TREATMENT OF VASCULAR DISEASE AND DIABETES THEREWITH

FIELD OF THE INVENTION

The present invention relates to 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt (1:1), 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt (1:1) hydrate, methods of producing 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt (1:1) in a crystalline form, methods of producing 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt (1:1) alcohol solvates in crystalline form, and the treatment of disease therewith. In particular, the 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt (1:1) and solvates thereof of the present invention are useful for lowering certain plasma lipids in animals including Lp(a), triglycerides, VLDL-cholesterol, and LDL-cholesterol, as well as elevating HDL cholesterol.

BACKGROUND OF THE INVENTION

Vascular diseases such as coronary heart disease, stroke, restenosis, and peripheral vascular disease, remain the leading cause of death and disability throughout the world. About 1.5 million people die each year in the United States alone from myocardial infarction resulting from congestive heart failure. While diet and life style can accelerate the onset of vascular diseases, genetic predisposition leading to dyslipidemia is a significant factor in vascular-related disabilities and deaths. "Dyslipidemia" means abnormal levels of lipoproteins in blood plasma.

Several risk factors have been associated with increased risk of vascular disease. Among these are the dyslipidemias of high levels of low-density lipoprotein (LDL) and low levels of high-density lipoproteins (HDL). The ratio of

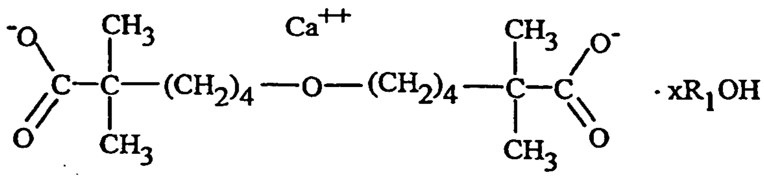
HDL-cholesterol to LDL-cholesterol is often used to assess the risk of vascular disease. A high ratio of HDL/LDL cholesterol is desirable.

Compounds that increase this ratio by either lowering LDL or increasing HDL, or both, therefore are beneficial. Studies have shown that elevated levels of a modified form of LDL designated as lipoprotein(a), "Lp(a)," are detrimental. Lp(a)-cholesterol appears to be undesirable, since elevated levels of Lp(a) have been associated with the development of atherosclerosis, coronary heart disease, myocardial infarction, stroke, cerebral infarction, and restenosis following balloon angioplasty. In fact, Lp(a) appears to be an excellent predictor of stroke potential. Accordingly, high concentrations of cholesterol in the form of Lp(a) are one of the major factors leading to death from heart disease.

US Patent No. 5,648,387 discloses the effectiveness of dialkyl ethers terminated with carboxylic acid, aldehyde, tetrazole, and esters of carboxylic acid in lowering plasma concentrations of Lp(a). The formation of pharmaceutically acceptable salts from the carboxylic acids is also contemplated by reaction with bases including sodium hydroxide, potassium hydroxide, calcium hydroxide, sodium carbonate, triethylamine, pyridine, and ammonia. Owing to the low melting character of the carboxylic acids and the lack of crystallinity and hydroscopic nature of the contemplated salts thereof, drying and crystallization of large quantities such as mass production lots remains inconsistent. Thus, there exists a need for a salt of the carboxyalkyl ether which is effective in raising HDL, lowering plasma Lp(a), which is crystalline and which is amenable to pharmaceutical formulation for the treatment of vascular disease.

SUMMARY OF THE INVENTION

This invention provides new chemical compounds, which are calcium dicarboxylate ethers. The invention more particularly provides compounds of the formula:



CI-1027
PD 0072953-0038
$C_{16}H_{28}O_5Ca \cdot xR_1OH$
$MW = 340.47 \cdot xR_1OH$

R_1 is H or lower alkyl inclusive of methyl, ethyl, 1-propyl, 2-propyl, 1-butyl
(II) = R_1 as H.

A method of drying the calcium salt from organic alcohols is provided. A method of crystallizing the monocalcium salt is provided.

A method of synthesizing such a monocalcium dicarboxylate ether salt is provided. The method includes exposing the dialkanoic ether acid to calcium oxide as the base in an organic solvent. After allowing sufficient time for the reaction to occur, a solid product is removed and dried to yield a calcium dicarboxylate ether salt having a stoichiometric ratio of calcium to dicarboxylate ether of 1:1 solvated with R_1OH. The R_1OH solvate can be removed by drying with humidification of the drying chamber in vacuo. The calcium dicarboxylate ether salt having a stoichiometric ratio of calcium to dicarboxylate ether of 1:1 can be crystallized by heating with water/water vapor at between 50°C to 150°C under pressure with agitation followed by vacuum drying. A second crystalline form can be obtained by heating the first form in water for an extended period of time. The compounds of the present invention are operative as active ingredients in combination with pharmaceutically acceptable diluents, carriers, and excipients to treat vascular disease. The use of calcium dicarboxylate ether salt or alcohol solvate for the manufacture of a compound for the treatment of vascular disease is also described within. The use of calcium dicarboxylate ether salt or alcohol solvate for the preparation of a compound for the treatment of diabetes is also described within.

BRIEF DESCRIPTION OF THE DRAWINGS

Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

Figure 1 is a two-dimensional drawing of the x-ray powder diffractogram of dicarboxylate ether monocalcium salt;

Figure 2 is a two-dimensional drawing of the x-ray powder diffractogram of the ethyl alcohol solvate of dicarboxylate ether monocalcium salt;

Figure 3 is a two-dimensional drawing of the x-ray powder diffractogram of II after humidification and drying of the ethyl alcohol solvate of dicarboxylate ether monocalcium salt;

Figure 4 is a two-dimensional drawing of the x-ray powder diffractogram of the methyl alcohol solvate of dicarboxylate ether monocalcium salt;

Figure 5 is a two-dimensional drawing of the x-ray powder diffractogram of II after humidification and drying of the methyl alcohol solvate of dicarboxylate ether monocalcium salt;

Figure 6 is a two-dimensional drawing of the x-ray powder diffractogram of the 1-propyl alcohol solvate of dicarboxylate ether monocalcium salt;

Figure 7 is a two-dimensional drawing of the x-ray powder diffractogram of II after humidification and drying of the 1-propyl alcohol solvate of dicarboxylate ether monocalcium salt;

Figure 8 is a two-dimensional drawing of the x-ray powder diffractogram of the 2-propyl alcohol solvate of dicarboxylate ether monocalcium salt;

Figure 9 is a two-dimensional drawing of the x-ray powder diffractogram of a compound of Formula II after humidification and drying of the 2-propyl alcohol solvate of dicarboxylate ether monocalcium salt;

Figure 10 is a two-dimensional drawing of the x-ray powder diffractogram of the 1-butyl alcohol solvate of dicarboxylate ether monocalcium salt;

Figure 11 is a two-dimensional drawing of the x-ray powder diffractogram of a compound of Formula II after humidification and drying of the 1-butyl alcohol solvate of dicarboxylate ether monocalcium salt;

Figure 12 is a three-dimensional comparison of x-ray powder diffractograms of the (a) methyl alcohol, (b) ethyl alcohol, (c) 1-propyl alcohol, (d) 2-propyl alcohol, and (e) 1-butyl alcohol solvates of the crystalline compounds of the present invention;

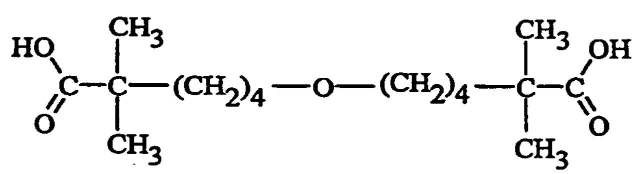
Figure 13 is a three-dimensional comparison of x-ray powder diffractograms of the effectively organic solvent free form of the compound according to the present invention derived from the solvates (a)-(e) depicted in Figure 12;

Figure 14 is a two-dimensional overlay of the x-ray diffractograms of organic solvent free 6-(5-carboxy-5-methyloxy)-2,2-dimethylhexanoic acid monocalcium salt produced from various alcohol solvates; and

Figure 15 is a two-dimensional drawing of the x-ray diffractogram of the crystalline form 2 after heating with water and isolation and drying.

DETAILED DESCRIPTION OF THE INVENTION

The synthesis of the precursor dialkylcarboxylic acid ether of the present invention is detailed in US Patent No. 5,648,387. The precursor dialkylcarboxylic acid ether of the present invention has the formula:



(I)

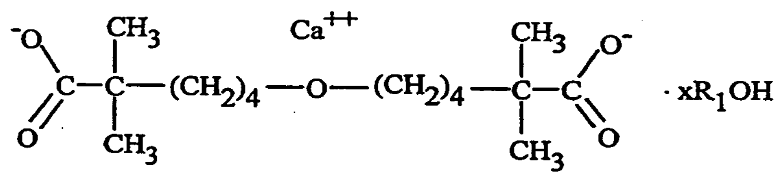
PD 0072953
$C_{16}H_{30}O_5$
MW = 302.41

To the precursor dialkylcarboxylic acid ether (I) is added calcium oxide. A small percentage of water can be present in the calcium oxide (up to 5%).

The acid base reaction of the present invention is preferably carried out in a solvent which dissolves the dialkylcarboxylic acid ether (I) and is at least only minimally reactive towards the calcium oxide. Preferably the calcium oxide partially dissolves in the solvent. Solvents operative in the present invention

illustratively include C_1-C_{12} alcohols including methyl alcohol, ethyl alcohol, 1-propyl alcohol, 2-propyl alcohol, butanols, pentanols, cyclopentanol, hexanols, cyclohexanol, and the like. Preferably, the solvent is a C_1-C_6 absolute alcohol.

The reaction of dialkylcarboxylic acid ether (I) with calcium oxide occurs at ambient or higher pressure and a temperature of greater than 25°C. However, it is appreciated that the reaction is facilitated by heating the reaction mixture to the reflux point of the solvent, or higher under pressure. Agitation further promotes uniform reaction throughout the reaction mixture. In order to assure conversion of most of the dialkylcarboxylic acid ether (I) to the mono-calcium salt, the molar ratio of calcium oxide to dialkylcarboxylic acid ether (I) should be between approximately 0.95 to approximately 1.05 molar equivalents. After allowing sufficient time for the reaction to occur between the dialkylcarboxylic acid ether (I) and the calcium oxide, a solid product is recovered. Typically, the reaction is complete, in refluxing solvent, in from about 4 to about 96 hours. A compound of the present invention results having the following formula:



(II)

CI-1027
PD 0072953-0038
$C_{16}H_{28}O_5Ca \cdot xR_1OH$
$MW = 340.47 \cdot xR_1OH$

wherein R_1 is H or lower alkyl inclusive of methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, and $x>0$. Typically, the amount of water present ranges from about 3% to about 5%.

Optionally, following the reaction between the dialkylcarboxylic acid ether (I) and the calcium oxide, the reaction is worked-up through the addition of a second solvent. The second solvent is miscible with the reaction solvent such that any calcium salt dissolved in the reaction solvent tends to precipitate from the solvent mixture and any unreacted organic materials remain in solution. It is appreciated that cooling the original solvent system or the mixed solvent system

containing dialkylcarboxylic acid ether monocalcium salt (II) further induces precipitation. The identity of the work-up solvent is dictated, in part, by the identity of the reaction solvent. For example, in the case of the alcohols, methyl *tert*-butyl ether is a representative work-up solvent. Other work-up solvents can include diethyl ether, tetrahydrofuran, and C_5-C_{12} mixed alkanes. However, any work-up solvent in which the dialkylcarboxylic acid ether monocalcium salt (II) is insoluble and which can be readily removed by drying can be used. Upon filtering/centrifuging off the solid product, dialkylcarboxylic acid ether monocalcium salt (II), the salt (II) is optionally washed with the work-up solvent and is thereafter dried to remove the majority of the byproduct water and the solvent mixture. Drying is facilitated by heating the salt (II) to a temperature greater than room temperature and less than the decomposition temperature of the salt (II). Drying can be with hot air, heated inert gas, or in vacuo. Preferably, the salt (II) is heated to a temperature range from between about 60°C and about 100°C to dry the salt. More preferably, the salt is heated under vacuum to further facilitate removal of the volatile solvents.

Surprisingly, it was discovered that heating and agitating the amorphous form of dialkylcarboxylic acid ether monocalcium salt (II) not only removed volatile solvents but also caused the dialkylcarboxylic acid ether monocalcium salt (II) to become crystalline.

It was found that humidification in a vacuum tray dryer facilitated the further removal of the volatile solvent to yield the amorphous form of dialkylcarboxylic acid ether calcium salt (II). The humidification can occur before or after complete drying of the dialkylcarboxylic acid ether monocalcium salt (II). Preferably, the solid dialkylcarboxylic acid ether monocalcium salt (II) is exposed to a humidification process prior to complete drying in order to facilitate removal of the volatile solvents to below the desired limit and to promote crystallinity.

Thus, following partial drying of the salt in a heated vacuum chamber, water and water vapor is introduced to the partially dried dialkylcarboxylic acid ether monocalcium salt (II). Both drying operations are preferably done with agitation. After the humidification, vacuum is reapplied until the salt (II) attains a stable weight. The dialkylcarboxylic acid ether monocalcium salt (II) obtained

following a humidification process has a bulk density following tapping of between 0.3 gm/mL and 0.52 gm/mL with an average of about 0.41 gm/mL as compared to an average of 0.31 gm/mL for amorphous material.

Also, it was found, surprisingly, that heating a suspension of crystalline form 1 of the monocalcium salt in water for extended periods of time converts it to a second crystalline form, designated crystalline form 2. These forms are distinguishable from one another by their respective x-ray powder diffraction patterns.

Both crystalline forms of Formula (II) dried production are observably less capable of retaining an electrostatic charge than salt (II) dried without exposure to humidification. The superior crystallinity of dialkylcarboxylic acid ether monocalcium salt (II) following the humidification process and a final drying is indicated by the x-ray diffraction (XRD) analysis. X-ray powder diffractograms of solvated salts (II) are shown in Figure 12 (a)-(e) and indicate solvate formation within the solid product from methyl alcohol, ethyl alcohol, 1-propyl alcohol, 2-propyl alcohol, and 1-butanol, respectively. Additional analysis on the post-humidification dried dialkylcarboxylic acid ether monocalcium salt (II) is indicative of the formation of a salt which is associated with between 0.1 and 1 molar equivalent of water per equivalent of dialkylcarboxylic acid ether calcium salt (II).

A pharmacological profile of dialkylcarboxylic acid ether monocalcium salt (II), which is designated hereafter as CI-1027, is largely consistent with peroxisome proliferator-activated receptor-α (PPARα) activation. It is generally believed that the pharmacological actions of the so-called "fibrates" are due to their interactions with PPARα. However, whereas fibrates are very potent PPARα ligands, in vitro ligand assays demonstrate that CI-1027 is a very weak agonist to PPARα, δ, or any other known PPAR. Additionally, unlike fibrates, such as fenofibrate and bezafibrate, CI-1027 elevates HDL-cholesterol in chow-fed rats (Ref. 1, Auerbach et al.). The inability of fibrates to elevate HDL-cholesterol in rats has been described to a lack of binding of rat PPARα to the apo-A1 promoter (Ref. 2, Auerbach et al. report) and the induction of Reverb-α (Ref. 3, Auerbach et al. report) which may repress apoA-1 transcription.

A preferred embodiment of the present invention utilizes CI-1027 to prevent and treat noninsulin dependent diabetes mellitus and conditions associated therewith, the administration being as detailed herein.

The compounds of the present invention have utility in treating and preventing vascular disease and noninsulin dependent diabetes mellitus and associated conditions. A further embodiment of this invention is a method of treating vascular disease and diabetes comprising administrating to a mammal in need of treatment an effective amount of a compound of Formula (II). An "effective amount" is the dose required to treat or prevent the vascular disease or diabetes of the mammal. The compounds are typically administered at a dose of about 50 to about 5000 mg/day, more generally about 50 to 2000 mg/day. A commonly employed dosage is from 50 to 900 mg/day. These same dosage levels are employed for the treatment and prevention of vascular disease, as well as for specifically lowering levels of Lp(a) and elevating HDL-cholesterol, and for treating and preventing diabetes.

Further embodiments of this invention are pharmaceutical formulations comprising a compound of Formula (II) together with pharmaceutically acceptable excipients, carriers, or diluents. The compounds are formulated for convenient oral, parenteral, or rectal administration, with oral delivery being preferred. Typically pharmaceutical carriers and excipients utilized in oral formulations include lactose, sucrose, starches, such as cornstarch and potato starch; cellulose derivatives such as methyl and ethyl cellulose; gelatins; talc; oils such as vegetable oils, sesame oil, cottonseed oil; and glycols such as polyethylene glycol. Oral preparations typically are in the form of tablets, capsules, emulsions, solutions, and the like. Controlled release formulations, for example, using a polymeric matrix or an osmotic pump, or the like, are also utilized. Typical formulations contain from about 5% to 95% by weight of a compound of Formula (II) administered with the excipient or carrier. Flavoring agents such as cherry flavor or orange flavor are incorporated.

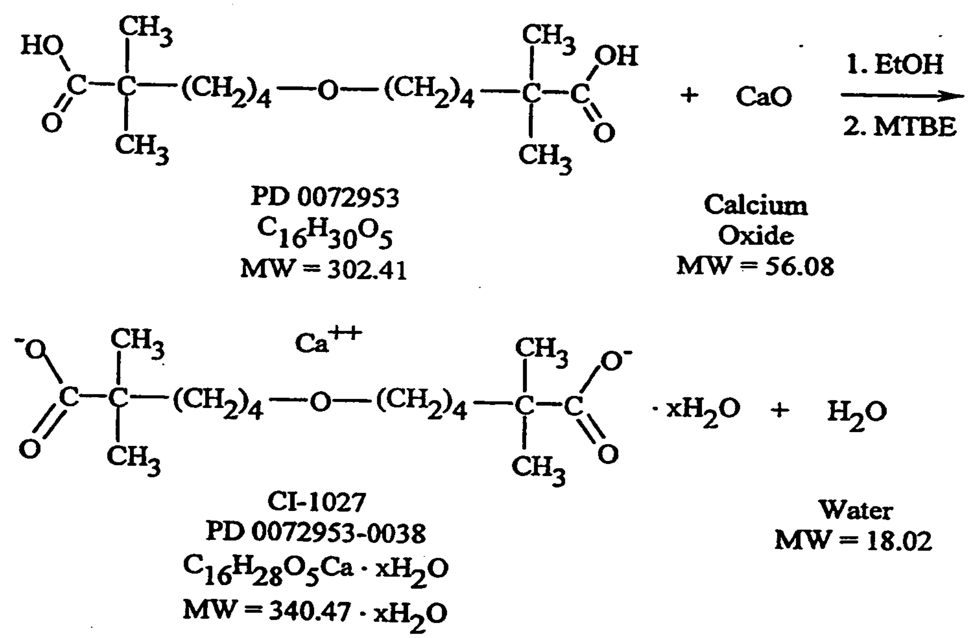
For parenteral administration, the compounds are optionally formulated with diluents such as isotonic saline, 5% aqueous glucose, and the like, for convenient intramuscular and intravenous delivery. The compounds optionally also are formulated with waxes and gels in the form of suppositories.

Examples 20-22 further illustrate typical formulations operative with the present invention.

In order to more fully demonstrate the advantages of the present invention, the following examples are set forth. It is to be understood that the following is by way of example only and should not be construed as a limitation on the scope of the present invention.

EXAMPLE 1

Preparation of 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt, Crystalline Form 1; CI-1027 (PD 0072953-0038)



PD 0072953
$C_{16}H_{30}O_5$
MW = 302.41

Calcium Oxide
MW = 56.08

CI-1027
PD 0072953-0038
$C_{16}H_{28}O_5Ca \cdot xH_2O$
MW = 340.47 · xH_2O

Water
MW = 18.02

Pilot Scale Example

Charge to 750 L glass-lined still:

6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid;

CI-1027 Step 1 (PD 0072953)—54.4 Kg, 179.9 mol

Calcium Oxide 98%—178.2 mol, 10.2 Kg

Ethyl Alcohol, pure anhydrous—392 Kg, 497 L

Start moderate agitation and heat mixture to reflux (76-80°C). Reflux reaction mixture for 96 hours. Cool to less than 50°C. Charge to reaction mixture:

Methyl tert-Butyl Ether—128 Kg, 163 L

Cool reaction mixture to 20°C to 25°C and stir approximately 1 hour. Filter solid product by centrifugation. Wash solid product with:

Methyl tert-Butyl Ethe—307 Kg, 391 L

Discharge product cake from centrifuge. Charge solvent wet product cake to 400 L hastelloy agitated pan dryer. Seal dryer and pull full vacuum (best available) on the system. Set jacket temperature for 85°C. Start agitation after approximately 1 hour at 85°C and full vacuum. Stir product at 85°C and full vacuum for >12 hours. Set jacket temperature to 100°C. Close valve to vacuum source. Charge by way of vacuum blank through injection nozzle:

Water, HPLC grade—6.4 Kg.

Water will vaporize and humidify the system. Stir the sealed, humidified system for 4 hours. Reapply vacuum and dry product for 18 to 24 hours. Cool system to below 30°C. Purge off vacuum with nitrogen. Discharge dry product from the dryer. Mill dry product through a Fitzmill with a #1A screen. White solid. Overall yield: 55.2 Kg (uncorrected for 4.04% water), 90.9%.

Analytical Results:

Identification (IR)—Consistent with structure.

Identification (^{1}H NMR)—Consistent with structure.

Identification (HPLC retention time)—Agrees with ARS-lot S.

Assay (HPLC wt/wt %)—99.30%

Ethyl Alcohol Content (wt % VPC)—0.06%

Water Content (TGA)—3.45%

Calcium Content (ICP, corrected for water)—12.91%

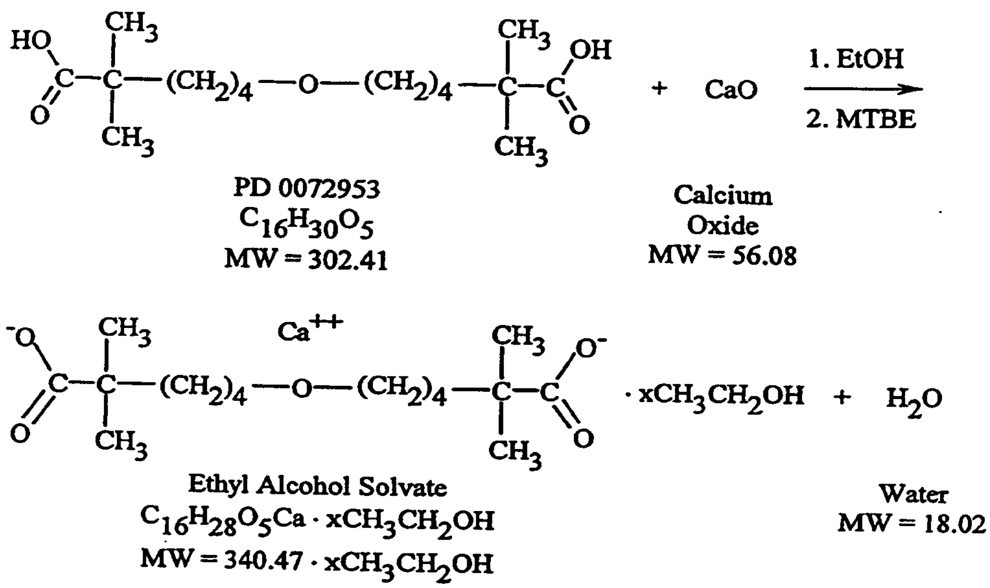
XRD—Crystalline Form 1, see Figure 1

^{13}C NMR (solid state) in ppm 189.6; 186.2; 71.4; 43.4; 30.1; 28.4; 25.2*; 23.1

The * indicates a resonance considered unique for this form by contract laboratory; SSCI, Inc. West Lafayette, Indiana.

EXAMPLE 2

Preparation of Crystalline 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt, ethyl alcohol solvate.



Standard Laboratory Method

Charge to 500 mL, 3-neck, round bottom with heating mantle, reflux condenser, and overhead stirring:

6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid;
CI-1027 Step 1 (PD 0072953)-25.0 g, 0.08267 mol

Calcium Oxide 98%—1.0 equivalent, 0.08267 mol, 4.73 g (corrected for purity)

Ethyl Alcohol—187.5 g, 237 mL

Start moderate agitation and heat mixture to reflux (76-80°C). Reflux reaction mixture for 4 to 24 hours. Cool to less than 50°C. Charge to reaction mixture:

Methyl tert-Butyl Ether—60.0 g, 79.2 mL

Cool reaction mixture to 20°C to 25°C and stir approximately 1 hour. Filter off solid product. Wash solid product with:

Methyl tert-Butyl Ether—40.0 g, 50 mL

Dry product at 60°C to 100°C and full vacuum to constant weight. Discharge from dryer. White solid. Overall yield: 21 to 30 g, 20 to 26 g dry basis (corrected for water and ethyl alcohol content), 80% to 95%.

<u>Experimental Ranges</u>

Calcium Oxide charge range examined (as 98%): 0.5 to 1.5 equivalents, 0.04134 to 0.1240 mol, 2.32 to 7.10 g

Calcium Oxide purities examined: 98-99.9% metallic purity

Ethyl Alcohol charge range examined: 5.0 to 10.0 g/g PD 0072953, 125 to 250 g, 158 to 316 mL

Reflux time ranges: 4 to 24 hours

Analytical Results:

Identification (IR)—Consistent with ARS-lot S

Identification (^{1}H NMR)—Consistent with ARS-lot S

Identification (^{13}C NMR)—Consistent with ARS-lot S

HPLC (Area % CI-1027)—99.738%

Ethyl Alcohol Content (wt % VPC)—1.95%

Water Content (KF titration)—1.73% (avg. of 3)

Calcium Content (ICP, corrected for water)—10.82%

XRD—Crystalline solvate, see Figure 2

^{13}C NMR (solid state) in ppm 189.9; 186.7; 71.6; 58.5*; 43.2; 29.9; 23.5

The * indicates a resonance considered unique for this form by contract laboratory; SSCI, Inc. West Lafayette, Indiana.

EXAMPLE 3

Preparation of 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt, Crystalline Form 1; CI-1027 (PD 0072953-0038).

Standard Laboratory Method

Charge to jacketed 500 mL, 3-neck, round bottom with overhead stirrer, vacuum gauge, water injection nozzle, and external temperature bath: 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt; solvent wet, ethyl alcohol solvate, or dry prepared from Standard Laboratory Method.

Seal reactor and start agitation (60-100 rpm). Pull full vacuum (best available) on the system. Set jacket temperature for 100°C. Stir product at 100°C and full vacuum for >2 hours. Close valve to vacuum source. Charge by way of vacuum blank through injection nozzle: Water—10 g.

Water will vaporize and humidify the system. Stir the sealed, humidified system for >30 minutes. Reapply vacuum and dry product for >3 hours. Cool system to below 30°C. Purge off vacuum with nitrogen. Discharge dry product from the reactor. White solid. Overall yield: 21 to 30 g, 20 to 26 g dry basis (corrected for water), 80% to 95%.

Experimental Ranges

Humidification/Drying Temperature range examined: 60°C to 100°C

Humidification Time range examined: 15 to 240 minutes

Water amount (humidification): 0 to 80 wt % (based on PD 0072953 charge), 0 to 20 g

Analytical Results

Identification (IR)—Consistent with ARS-lot S

Identification (^{1}H NMR)—Consistent with ARS-lot S

Identification (^{13}C NMR)—Consistent with ARS-lot S

HPLC (Area % CI-1027)—99.725%

Ethyl Alcohol Content (wt % VPC)—0.0%

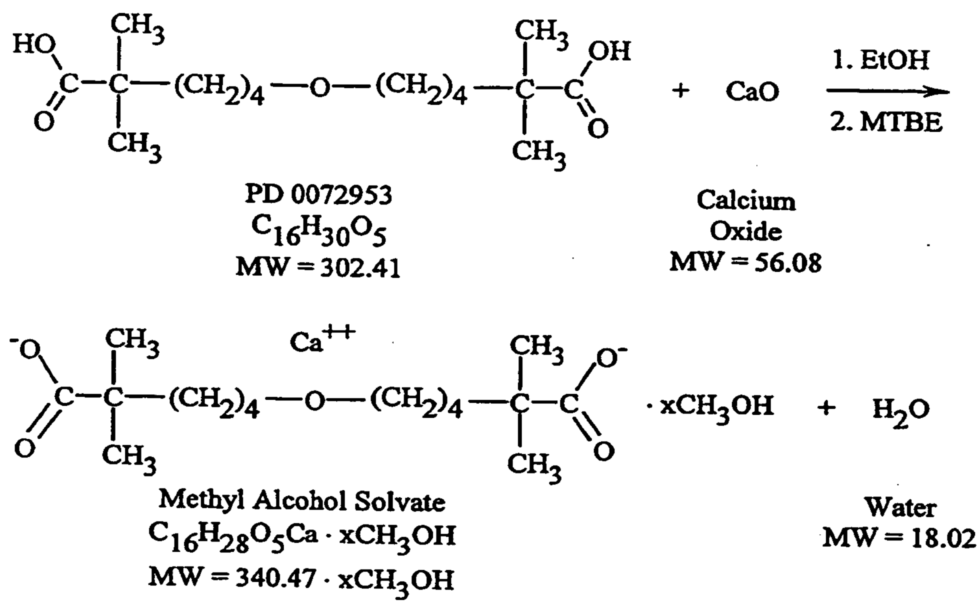
Water Content (KF titration)—3.25%

Calcium Content (ICP, corrected for water)—NA

XRD—Crystalline Form 1, see Figure 3

EXAMPLE 4

Preparation of Crystalline 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt, methyl alcohol solvate.



Standard Laboratory Method

Charge to 500 mL, 3-neck, round bottom with heating mantle, reflux condenser, and overhead stirring:

6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid;

CI-1027 Step 1 (PD 0072953) – 25.0 g, 0.08267 mol

Calcium Oxide 98%—1.0 equivalent, 0.08267 mol, 4.73 g (corrected for purity)

Start moderate agitation and heat mixture to reflux (76-80°C). Reflux reaction mixture for 24 hours. Cool to less than 50°C. Charge to reaction mixture:

Methyl tert-Butyl Ether—60.0 g, 79.2 mL

Cool reaction mixture to 20°C to 25°C and stir approximately 1 hour. Filter off solid product. Wash solid product with:

Methyl tert-Butyl Ether —40.0 g, 50 mL

Dry product at 60°C to 100°C and full vacuum to constant weight. Discharge from dryer. White solid. Overall yield: 21 to 30 g, 20 to 26 g dry basis (corrected for water and methyl alcohol content), 80% to 95%.

Analytical Results:

Identification (IR)—Consistent with ARS-lot S

Identification (^{1}H NMR)—Consistent with ARS-lot S

Identification (^{13}C NMR)—Consistent with ARS-lot S

HPLC (Area % CI-1027)—99.737%

Methyl Alcohol Content (wt % VPC)—NA

Water Content (KF titration)—3.36% to 4.94% (range of 3)

Calcium Content (ICP, corrected for water)—11.00% to 11.22% (water range)

XRD—Crystalline solvate, see Figure 4

^{13}C NMR (solid state) in ppm: 189.6; 186.2; 71.4; 43.2; 29.6; 23.5

EXAMPLE 5

Preparation of 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt, Crystalline Form 1 from methyl alcohol solvate; CI-1027 (PD 0072953-0038)

Standard Laboratory Method

Charge to jacketed 500 mL, 3-neck, round bottom with overhead stirrer, vacuum gauge, water injection nozzle, and external temperature bath:

6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt; methyl alcohol solvate, prepared from Standard Laboratory Method.

Seal reactor and start agitation (60-100 rpm). Pull full vacuum (best available) on the system. Set jacket temperature for 100°C. Stir product at 100°C and full vacuum for >2 hours. Close valve to vacuum source. Charge by way of vacuum blank through injection nozzle: Water—10 g.

Water will vaporize and humidify the system. Stir the sealed, humidified system for >30 minutes. Reapply vacuum and dry product for >3 hours. Cool system to below 30°C. Purge off vacuum with nitrogen. Discharge dry

product from the reactor. White solid. Overall yield: 21 to 30 g, 20 to 26 g dry basis (corrected for water), 80% to 95%.

Analytical Results:

Identification (IR)—Consistent with ARS-lot S

Identification (^{1}H NMR)—Consistent with ARS-lot S

Identification (^{13}C NMR)—Consistent with ARS-lot S

HPLC (Area % CI-1027)—99.24%

Ethyl Alcohol Content (wt % VPC)—0.0%

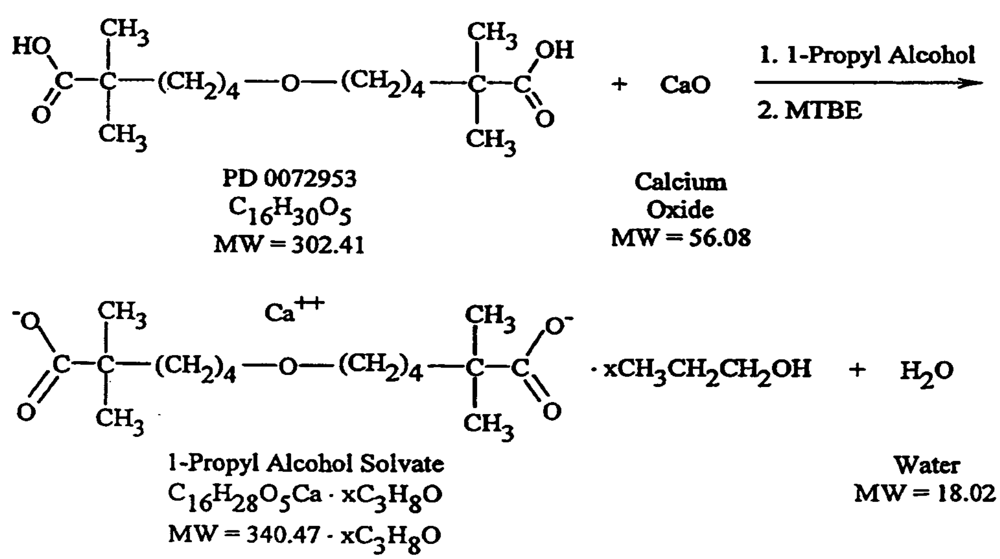
Water Content (KF titration)—3.84%

Calcium Content (ICP, corrected for water)—11.52%

XRD—Crystalline form, see Figure 5.

EXAMPLE 6

Preparation of Crystalline 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt, 1-propyl alcohol solvate



Standard Laboratory Method

Charge to 500 mL, 3-neck, round bottom with heating mantle, reflux condenser, and overhead stirring:

6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid;

CI-1027 Step 1 (PD 0072953)—25.0 g, 0.08267 mol

Calcium Oxide 98%— 1.0 equivalent, 0.08267 mol, 4.73 g (corrected for purity)

1-Propyl Alcohol— 187.5 g, 233 mL

Start moderate agitation and heat mixture to reflux (76-80°C). Reflux reaction mixture for 24 hours. Cool to less than 50°C. Charge to reaction mixture:

Methyl tert-Butyl Ether—60.0 g, 79.2 mL

Cool reaction mixture to 20°C to 25°C and stir approximately 1 hour. Filter off solid product. Wash solid product with:

Methyl tert-Butyl Ether—40.0 g, 50 mL

Dry product at 60°C to 100°C and full vacuum to constant weight. Discharge from dryer. White solid. Overall yield: 21 to 30 g, 20 to 26 g dry basis (corrected for water and 1-propyl alcohol content), 80% to 95%.

Analytical Results:

Identification (IR)—Consistent with ARS-lot S

Identification (^{1}H NMR)—Consistent with ARS-lot S. 1-Propyl alcohol present.

Identification (^{13}C NMR)—Consistent with ARS-lot S. 1-Propyl alcohol present

HPLC (Area % CI-1027)—99.064%

1-Propyl Alcohol Content (TGA)—5.99%

Water Content (KF titration)—1.72% (avg. of 3)

Calcium Content (ICP, corrected for water)—10.73%

XRD—Crystalline solvate, see Figure 6

^{13}C NMR (solid state) in ppm 189.9; 186.0; 71.6; 43.2; 29.6; 23.8

EXAMPLE 7

Preparation of 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt, Crystalline Form 1 from 1-propyl alcohol solvate; CI-1027 (PD 0072953-0038)

Standard Laboratory Method

Charge to jacketed 500 mL, 3-neck, round bottom with overhead stirrer, vacuum gauge, water injection nozzle, and external temperature bath:

6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt; CI-1027 (PD 0072953-0038, 1-propyl alcohol solvate, prepared from Standard Laboratory Method.

Seal reactor and start agitation (60-100 rpm). Pull full vacuum (best available) on the system. Set jacket temperature for 100°C. Stir product at 100°C and full vacuum for >2 hours. Close valve to vacuum source. Charge by way of vacuum blank through injection nozzle: Water—10 g.

Water will vaporize and humidify the system. Stir the sealed, humidified system for >30 minutes. Reapply vacuum and dry product for >3 hours. Cool system to below 30°C. Purge off vacuum with nitrogen. Discharge dry product from the reactor. White solid. Overall yield: 21 to 30 g, 20 to 26 g dry basis (corrected for water), 80% to 95%.

Analytical Results:

Identification (IR)—Consistent with ARS-lot S

Identification (^{1}H NMR)—Consistent with ARS-lot S

Identification (^{13}C NMR)—Consistent with ARS-lot S

HPLC (Area % CI-1027)—99.519%

1-Propyl Alcohol Content (TGA)—0.0%

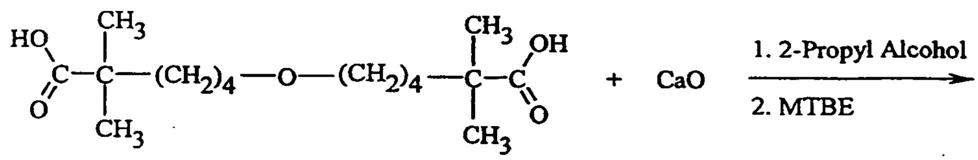
Water Content (KF titration)—3.98% (avg. of 2)

Calcium Content (ICP, corrected for water)—10.20%

XRD—Crystalline form, see Figure 7.

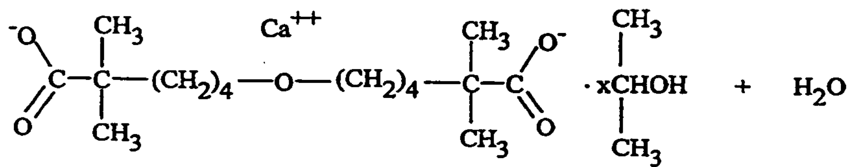
EXAMPLE 8

Preparation of Crystalline 6-(5-carboxy-5-methyl-hexyl xy)-2,2-dimethylhexanoic acid monocalcium salt, 2-propyl alcohol solvate



PD 0072953
$C_{16}H_{30}O_5$
MW = 302.41

Calcium Oxide
MW = 56.08



2-Propyl Alcohol Solvate
$C_{16}H_{28}O_5Ca \cdot xC_3H_8O$
MW = 340.47 · xC_3H_8O

Water
MW = 18.02

Standard Laboratory Method

Charge to 500 mL, 3-neck, round bottom with heating mantle, reflux condenser, and overhead stirring:

6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid;
CI-1027 Step1 (PD 0072953)—25.0 g, 0.08267 mol

Calcium Oxide 98%—1.0 equivalent, 0.08267 mol, 4.73 g (corrected for purity)

2-Propyl Alcohol—187.5 g, 239 mL

Start moderate agitation and heat mixture to reflux (76-80°C). Reflux reaction mixture for 24 hours. Cool to less than 50°C. Charge to reaction mixture:

Methyl tert-Butyl Ether—60.0 g, 79.2 mL

Cool reaction mixture to 20°C to 25°C and stir approximately 1 hour. Filter off solid product. Wash solid product with:

Methyl tert-Butyl Ether—40.0 g, 50 mL

Dry product at 60°C to 100°C and full vacuum to constant weight. Discharge from dryer. White solid. Overall yield: 21 to 30 g, 20 to 26 g dry basis (corrected for water and 2-propyl alcohol content), 80% to 95%.

Analytical Results:

Identification (IR)—Consistent with ARS-lot S

Identification (^{1}H NMR)—Consistent with ARS-lot S. 2-Propyl alcohol present.

Identification (^{13}C NMR)—Consistent with ARS-lot S. 2-Propyl alcohol present.

HPLC (Area % CI-1027)—99.315%

2-Propyl Alcohol Content (TGA)—6.12%

Water Content (KF titration)—1.96% (avg. of 3)

Calcium Content (ICP, corrected for water)—10.27%

XRD—Crystalline solvate, see Figure 8.

EXAMPLE 9

Preparation of 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt, Crystalline Form 1 from 2-propyl alcohol solvate; CI-1027 (PD 0072953-0038)

Standard Laboratory Method

Charge to jacketed 500 mL 3-neck round bottom with overhead stirrer, vacuum gauge, water injection nozzle, and external temperature bath:

6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt; 2-propyl alcohol solvate, prepared from Standard Laboratory Method.

Seal reactor and start agitation (60-100 rpm). Pull full vacuum (best available) on the system. Set jacket temperature for 100°C. Stir product at 100°C and full vacuum for >2 hours. Close valve to vacuum source. Charge by way of vacuum blank through injection nozzle: Water—10 g.

Water will vaporize and humidify the system. Stir the sealed, humidified system for >30 minutes. Reapply vacuum and dry product for >3 hours. Cool system to below 30°C. Purge off vacuum with nitrogen. Discharge dry product from the reactor. White solid. Overall yield: 21 to 30 g, 20 to 26 g dry basis (corrected for water), 80% to 95%.

Analytical Results:

Identification (IR)—Consistent with ARS-lot S

Identification (^{1}H NMR)—Consistent with ARS-lot S

Identification (^{13}C NMR)—Consistent with ARS-lot S

HPLC (Area % CI-1027)—99.611%

2-Propyl Alcohol Content (TGA)—0.0%

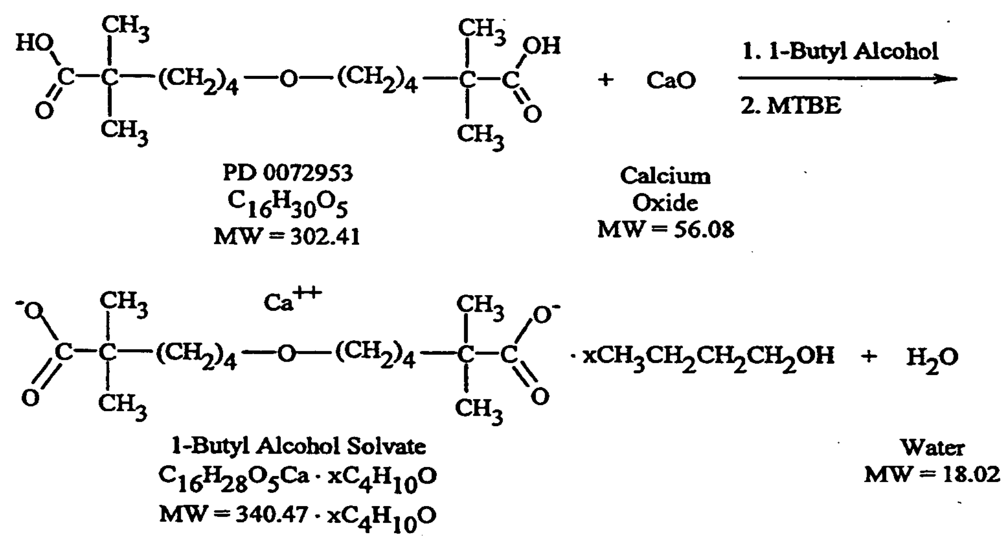
Water Content (KF titration)—4.04% (avg. of 2)

Calcium Content (ICP, corrected for water)—10.93%

XRD—Crystalline solvate, see Figure 9.

EXAMPLE 10

Preparation of Crystalline 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt, 1-butyl alcohol solvate



Standard Laboratory Method

Charge to 500 mL, 3-neck, round bottom with heating mantle, reflux condenser, and overhead stirring:

6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid;

CI-1027 Step 1 (PD 0072953) – 25.0 g, 0.08267 moles

Calcium Oxide 98%—1.0 equivalent, 0.08267 mol, 4.73 g (corrected for purity)

1-Butyl Alcohol—187.5 g, 231.5 mL

Start moderate agitation and heat mixture to reflux (76-80°C). Reflux reaction mixture for 24 hours. Cool to less than 50°C. Charge to reaction mixture:

Methyl tert-Butyl Ether—60.0 g, 79.2 mL

Cool reaction mixture to 20°C to 25°C and stir approximately 1 hour. Filter off solid product. Wash solid product with: Methyl tert-Butyl Ether—40.0 g, 50 mL.

Dry product at 60°C to 100°C and full vacuum to constant weight. Discharge from dryer. White solid. Overall yield: 21 to 30 g, 20 to 26 g dry basis (corrected for water and n-butyl alcohol content), 80% to 95%.

Analytical Results:

Identification (IR)—Consistent with ARS-lot S

Identification (^{1}H NMR)—Consistent with ARS-lot S. N-Butyl alcohol present.

Identification (^{13}C NMR)—Consistent with ARS-lot S. N-Butyl alcohol present.

HPLC (Area % CI-1027) —99.560%

1-Butyl Alcohol Content (TGA)—9.02%

Water Content (KF titration)—1.93% (avg. of 2)

Calcium Content (ICP, corrected for water)—9.65%

XRD—Crystalline solvate, see Figure 10

^{13}C NMR (solid state) in ppm 189.9; 186.0; 71.6; 43.2; 29.9; 23.8

EXAMPLE 11

Preparation of 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt, Crystalline Form 1 from 1-butyl alcohol solvate; CI-1027 (PD 0072953-0038)

Standard Laboratory Method

Charge to jacketed 500 mL, 3-neck, round bottom with overhead stirrer, vacuum gauge, water injection nozzle, and external temperature bath:

6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt; 1-butyl alcohol solvate, prepared from Standard Laboratory Method

Seal reactor and start agitation (60-100 rpm). Pull full vacuum (best available) on the system. Set jacket temperature for 100°C. Stir product at 100°C and full vacuum for >2 hours. Close valve to vacuum source. Charge by way of vacuum blank through injection nozzle: Water—10 g.

Water will vaporize and humidify the system. Stir the sealed, humidified system for >30 minutes. Reapply vacuum and dry product for >3 hours. Cool system to below 30°C. Purge off vacuum with nitrogen. Discharge dry product from the reactor. White solid. Overall yield: 21 to 30 g, 20 to 26 g dry basis (corrected for water), 80% to 95%.

Analytical Results:

Identification (IR)—Consistent with ARS-lot S

Identification (^{1}H NMR)—Consistent with ARS-lot S

Identification (^{13}C NMR)—Consistent with ARS-lot S

HPLC (Area % CI-1027)—99.374%

1-Butyl Alcohol Content (TGA)—0.0%

Water Content (KF titration)—3.96% (avg. of 3)

Calcium Content (ICP, corrected for water)—10.70%

XRD—Crystalline, see Figure 11.

EXAMPLE 12

Preparation of 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt with water. Crystalline Form 2 formed by water digestion; CI-1027 (PD 0072953-0038)

Standard Laboratory Method

Charge to 200 mL round bottom flask:

6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt; CI-1027 (PD 0072953-0038, Crystalline Form 1 prepared using Pilot Plant Example method—24.4 g

Water—100 g

The round bottom flask containing the slurry was attached to a rotary evaporator and a slow rotation initiated (120 rpm). The round bottom containing the slurry was then immersed in a water bath set for a temperature of 60°C. The system was mixed under atmospheric pressure for 7 days.

Cool to 20°C to 25°C. Filter off solid product. Wash solid product with: Water—50 g

Dry product at 90°C and full vacuum to constant weight. Discharge from dryer. White solid. Overall yield: 21.3 g, 20.6 g dry basis (corrected for water), 84%.

Analytical Results:

Identification (IR)—Consistent with ARS-lot S*

Identification (^{1}H NMR)—Consistent with ARS-lot S*

Identification (^{13}C NMR) —NA

HPLC (Area % CI-1027)—100.06%*

Ethyl Alcohol Content (wt % VPC)—0.04%*

Water Content (KF titration)—3.47%

Calcium Content (ICP, corrected for water)—10.78%

XRD—Crystalline Form, see Figure 15

^{13}C NMR (solid state): results pending

* Results from pilot scale lot used in the experiment. Assuming no changes in product structure, purity, or solvent content.

EXAMPLE 13

Comparison of monoalkali earth salts to other salts

In order to compare the properties of the monocalcium salts of the present invention to other salts, the preparation according to Example 1 was repeated with sodium hydroxide, potassium hydroxide, and acetylcholine in stoichiometric ratios of 1:1 per dialkanoic acid molecule and 2:1 per dialkanoic acid molecule. The properties of the solid as prepared and following complete drying are shown for the various salts in Table 1.

Table 1

Salt Form and Hygroscopic Status

Salt Prepared	Physical Form	Physical Properties of Solid
di-sodium	Solid	Hygroscopic
di-potassium	Solid	Hygroscopic
mono-choline	Oil	-
di-choline	Oil	-
mono-sodium	Solid	Hygroscopic
mono-potassium	Solid	Hygroscopic
mono-calcium	Solid	Slightly Hygroscopic

EXAMPLE 14

The effects of CI-1027 on Lp(a) and other lipoprotein parameters in two models of elevated Lp(a)

The cynomolgus macaque monkeys and Lp(a) transgenic mice are dosed with CI-1027 at 3, 10, 30, 100, or 300 mg/kg for 2 weeks by oral gavage. Lp(a) lowering is dose dependent (-9, -23, -64, -68, and -87% for the 3, 10, 30, 100, and 300 mg/kg/day doses, respectively). In these, studies total plasma and HDL cholesterol decreased. In the transgenic mouse study, female mice are allocated into five groups with equivalent Lp(a) levels, and dosed by oral gavage with either vehicle alone or vehicle plus CI-1027 (3, 10, 30, and 100 mg/kg/day). Blood is sampled weekly (2 weeks prior to treatment, 2 weeks on treatment). At the start of the study, plasma Lp(a) averaged 40 mg; 1dl across the groups. After 1 week, CI-1027 caused a dose dependent decrease in plasma Lp(a) (-15, -41, -54, and -61% for the 3, 10, 30, and 100 mg/kg/day dose levels, respectively) as compared to mice dosed with vehicle alone. There is also a dose-related decrease in total plasma, cholesterol, with a maximum decrease of 32% at the 100 mg/day dose. Lipoprotein profiles determined by HPLC demonstrated that the decrease in cholesterol is due primarily to significant decreases in LDL cholesterol. HDL cholesterol remained unchanged. The ratio of HDL cholesterol to VLDL + LDL cholesterol improved with treatment from a control value of 0.39 to 0.65. Plasma apoB was also

decreased by up to 30%. Changes are similar following the second week of treatment.

EXAMPLE 15

The effects of CI-1027 on insulin sensitivity

CI-1027 is evaluated in a standard assay utilizing 3T3-Ll adipocytes, which are particularly responsive to insulin, ie, sugar uptake can be acutely activated 15- to 20-fold by insulin. The methodology utilized for the assay is described more fully by Frost et al., *J Biol. Chem.*, 1985;260:2646-2652. Specifically, 3T3-Ll fibroblast cells were obtained from American Type Culture Collection (ATCC, Rockville, MD). Cells were grown to confluence and differentiated into adipocytes. On Day 0, confluent cells were treated with 167 mm insulin, 0.25 μM dexamethasone, and 0.5 mM methyl isobutylmethylxanthine in 10% fetal bovine serum (FBS) containing Dulbecco's Modified Eagle's Medium (DMEM). Two days later, the media was changed to DMEM containing 167 nm insulin and 10% FBS. The media was then switched to 10% DMEM and changed every other day until harvest. The experimental compounds, solubilized in dimethyl sulfoxide, were included in the media on Day 0 and replenished with each media change. Differentiation was assessed by visualizing the accumulation of fat droplets in the cells. Glucose transport was measured by quantitating the incorporation of [^{14}C]deoxyglucose in differentiated cells on Day 9, according to the methodology described by Sandouk, et al., *Endocrinology*, 1993;133:352-359.

EXAMPLE 16

Pharmacokinetics and metabolism of [^{14}C]CI-1027

CI-1027 is under clinical evaluation for the treatment of dyslipidemias and atherosclerosis by elevating high-density lipoprotein cholesterol (HDL-C) and lowering the atherogenic lipoprotein Lp(a). CI-1027 is rapidly absorbed in the rat, dog, and monkey. Oral bioavailability appeared to be high even though CI-1027 pharmacokinetics are nonlinear and the drug seemed to undergo enterohepatic recirculation. Apparent intravenous (IV) and per orals (PO) elimination half-life

values are shorter in rat (5 to 7 hours) than in dog (17 to 31 hours) or in the monkey (9 to 15 hours). In vitro binding to plasma proteins is species and concentration dependent. Albumin appeared to be the primary binding protein. In vitro studies with rat, dog, and monkey hepatocytes using radiolabeled compound revealed two major ^{14}C peaks, intact drug, and a glucuronide conjugate. Mean recovery (percent ^{14}C dose) in intact and bile-fistula cannulated rats and monkeys following 10 mg/kg [^{14}C] is shown below in Table 2.

Table 2

Mean Recovery as Percent of 10 mg/kg and ^{14}C dose

Excreta	Intact Rat	Fistula Rat	Monkey	Fistula Monkey
Bile		87.5		42.0
Urine	37.0	10.5	78.1	62.2
Feces	56.9	0.72	17.3	3.82
Total	93.9	98.7	95.4	108

Metabolite profiling is performed by HPLC with radiometric detection and metabolites arc identified by LC/RAM/MS/MS. Essentially 100% of the plasma radioactivity was unchanged drug. Since an acyl-glucuronide is detected in bile and urine, LC/NMR analysis is performed to examine the potential acyl-migration products.

EXAMPLE 17

Experimental animal protocols

Mice

Mice transgenic for human $apoB_{100}$ and apo(a) (24) were obtained by license agreement from Dr. Ed Rubin (IJC, Berkeley, CA). Mice were bred to homozygosity for each trait and then crossed to generate offspring with moderate (mean Lp(a) -35 mg/dL) levels of human Lp(a). Female mice were allocated into treatment groups based on pretreatment Lp(a) levels. All animals were allowed normal chow (Ralston-Purina) and water ad libitum in temperature controlled rooms, with a 12-hour light, 12-hour dark cycle beginning with lights on at 6:00 A.M. During the treatment phase of the study the mice were dosed daily between 6:00 and 9:00 A.M. by oral gavage using a suspension vehicle of 1.5%

carboxymethylcellulose plus 0.2% Tween-20 (CMC/Tween) containing CI-1027. Control animals received vehicle alone, vehicle volume represented 0.25% of body weight. Under anesthesia, tail blood was obtained weekly in the morning for 2 weeks prior to and after 1 and 2 weeks of drug or vehicle treatment.

<u>Monkeys</u>

Adult male and female, cynomolgus macaques were individually caged, in temperature controlled rooms, with a 12-hour light, 12-hour dark cycle. Animals were maintained on Purina Monkey Chow. Chair restrained monkeys were dosed with CI-1027 by oral gavage in a vehicle of 0.2% Tween 20/1.5% carboxymethylcellulose in water. Fasting blood samples were collected via surgically implanted intra-vascular ports.

EXAMPLE 18

Analytical methods of pharmacological effects

Plasma total cholesterol (Boehringer Mannheim, Indianapolis, IN) and, triglycerides (Wako Pure Chemicals, Richmond, VA) are determined enzymatically **(may need reference)**. Plasma lipoprotein cholesterol distributions are determined by high-performance gel-filtration chromatography with post-column detection **(may need reference)**. Human apoAI, apoB, and Lp(a) are assessed immunoturbidometrically utilizing commercially available kits (Wako Pure Chemicals, Richmond, VA) on a Cobas Mira Plus Analyzer (Roche Diagnostics, where?).

EXAMPLE 19

Pharmacokinetics (PK) and Pharmacodynamics (PD) of Orally Administered C1-1027 in Rats and Monkeys

Purpose. To assess CI-1027 PK/PD relationships in rats and cynomolgus monkeys following repeated oral (PO) administration. Previous preclinical studies have shown that CI-1027 elevates high-density lipoprotein cholesterol (HDL-C) in rats and lowers atherogenic apoB containing lipoproteins [Lp(a)] in monkeys,

CI-1027 is currently under clinical evaluation for the treatment of dyslipidemias and atherosclerosis.

Methods. Five groups of four male rats each were administered increasing once-daily gavage doses of Cl- 1027 (1, 3, 10, 30, or 100 mg/kg) for 14 days. Three groups of four monkeys (N=2/sex) each were administered increasing once-daily gavage doses of CI-1027 (3, 10, or 30 mg/kg) for 14 days. Serial blood samples were collected from rats and monkeys up to 24 hours after the last dose. Plasma was harvested and samples were analyzed for CI-1027 by a validated LC/MS/MS assay. PK parameters were calculated from plasma CI-1027 concentration-time data using noncompartmental methods. Plasma lipids (HDL-C in rats; Lp(a) in monkeys) were measured by routine clinical chemistry methods. Relationships between dose or PK (Cmax, AUC(0-24)) and PD effect (%change in HDL-C on Day 14 from controls or %change in average daily LP(a) ,on Day 14 from baseline) were examined using the traditional Emax or sigmoidal Emax models (WinNonlin 2.0).

Results. Mean CI-1027 Cmax and AUC(0-24) values increased more than proportionally with dose in rats and were dose proportional in monkeys. Modeling (Emax for rat data; sigmoidal Emax for monkey data) of observed PD effect (%change in HDL-C in rats, %change in Lp(a) in monkeys) versus daily dose (mg/kg) or PK [Cmax or AUC(0-24)] resulted in comparable fits, suggesting that observed effects are well-characterized by dose alone. In rats, plasma HDL-C concentrations increased in a dose dependent manner with an Emax of 172% at 100 mg/kg/day. In monkeys, plasma Lp(a) concentrations decreased in a dose dependent manner with an Emax of 64.5% at 30 mg/kg/day.

Conclusions. CI-1027 PK/PD data from rats and monkeys can be modeled using an Emax and sigmoidal Emax models, respectively. Multiple once daily gavage administration of CI-1027 results in a dose-dependent modulation of plasma lipids; in rats and monkeys.

EXAMPLE 20

Capsule formulation

Ingredient	Amount
6-(5-carboxy-5-methyl-hexyloxy)-2, 2-dimethylhexyanoic acid monocalcium salt	1000 g
Lactose	960 g
Magnesium Stearate	40 g

The ingredients are blended to uniformity and filled into #4 hard gelatin capsules. Each capsule is filled with 200 mg of the blended mixture and contains 100 mg of active monocalcium dicarboxylate ether. The capsules are administered to an adult human at the rate of one to three each day to lower plasma Lp(a).

EXAMPLE 21

Tablet formulation

Ingredient	Amount
6-(5-methyl-hexyloxy)-2,2-dimethyl-1-hexanoic acid monocalcium salt	3000 g
Lactose	750 g
Cornstarch	300 g
Gelatin	120 g
Water	1000 cc
Magnesium stearate	20 g

The dialkyl ether salt, lactose, and 150 g of the cornstarch are blended with a solution of the gelatin in the water. The wet granulation is screened, dried, and re-screened. The dried granules are blended with the magnesium stearate and the remaining cornstarch, and the mixture is compressed into 698 mg tablets using 15/32 inch standard concave punches. Each tablet contains 500 mg of dialkyl ether salt.

EXAMPLE 22

Oral liquid formulation

Ingredient	Amount
6-(5-carboxy-5-methyl-hexyloxy)-2, 2-dimethy1hexanoic acid monocalcium salt	4.0 g
Polyoxyethylene sorbital monostearate	0.1 cc
Sodium carboxymethyl cellulose	0.3 g
Complex Magnesium Aluminum Silicate	0.5 g
Sugar	10 g
Glycerin	2 cc
Sodium benzoate	0.5 g
Sodium citrate	0.2 g
Approved red dye	1 mg
Cherry flavor	0.02 cc
Distilled water qs	100 cc

The polyoxyethylene sorbital monostearate is a product such as polysorbate 60 or Tween 60. The complex magnesium-aluminum silicate is a gel-forming agent, such as Vcegum H.V. This substance is hydrated overnight in 10 cc of distilled water. A mixture is prepared from the polyoxyethylene sorbital monostearate, imitation cherry flavor, 30 cc of distilled water, and the alkaline earth dicarboxylate ether and passed through a homogenizer. With vigorous stirring, the sugar, glycerin sodium citrate, sodium benzoate, and sodium carboxymethylcellulose are added, followed by hydrated complex magnesium-aluminum silicate and a solution of the red dye in 2 cc of water. The resulting suspension is homogenized, adjusted to pH 5.0 with citric acid, and diluted to a final volume of 100 cc with distilled water. A 55-cc oral dosage unit of this suspension contains 100 mg of the dialkyl acid ether salt. If desired, the red dye and imitation cherry flavor can be omitted or replaced by other coloring and flavoring agents.

EXAMPLE 23

Pilot Scale Data

Upon scale-up of the CI-1027 process, difficulties were encountered in drying the final product. The monocalcium salt is formed in refluxing ethyl alcohol. The removal of the ethyl alcohol from the isolated solid product proved very difficult at scale using typical maximum drying conditions (100°C, full vacuum) and vacuum tray dryers. Different types of agitated dryers were investigated without success. Although some small-scale lots were dried to acceptable levels of ethyl alcohol, the results were inconsistent and the conditions applied were not conducive to further scale-up. See Table A for drying examples.

Viable methods of drying this product at a large scale were investigated in our process development effort. It was discovered that the exposure of the product to humidity greatly accelerated the removal rate of the ethyl alcohol. This method was initially applied to the vacuum tray dryers with some success. The further application to agitated pan dryers resulted in a process whereby the ethyl alcohol was easily removed in a short time period. This drying process produced consistent product in short cycle times and therefore demonstrates large-scale potential.

The initial drying method without the use of humidity produced a product with an amorphous physical form by x-ray diffraction Examples CD-2969C through CD3102 (excluding CD-3082) in Table A. The humidification process in the agitated pan dryer produced a product with a crystalline form by x-ray diffraction. Subsequently, it was observed that the crystalline form exhibited some advantages over the amorphous form. The crystalline form has a higher bulk density that has been reasonably consistent upon scale-up as shown in Table B. The bulk density of the amorphous form was observed to be decreasing with scale. The crystalline form is also observably less electrostatic which improves the handling characteristics of the bulk drug. It should be noted that solvent free amorphous product can be converted to product with a crystalline form with humidification. Solvent content is not required for the conversion.

The discovery of the crystalline form prompted further research into the mechanism of formation. This research led to the discovery of a series of stable, crystalline solvates of the final product mono-calcium salt. These alcohol solvates

exhibit distinct crystalline forms by x-ray diffraction and are stable under conditions of greater than 60°C and full vacuum. It should be noted that the solvates are easily converted to solvent free crystalline final product with the same x-ray diffraction pattern using the humidification process.

In summary, the advantages of the humidification process and resultant crystalline product are as follows:

1. The humidification process allows for the effective drying of the final product at a large scale. It produces a consistent, solvent free (below current specification of 0.5% ethyl alcohol) product in a much shorter time period.
2. The resultant crystalline, solvent free product generally exhibits a higher bulk density than the amorphous product. This bulk density has been reasonably consistent upon scale-up whereas the amorphous bulk density was observed to drop upon scale-up.
3. The resultant crystalline, solvent free product is observably less electrostatic than the amorphous product. This greatly improves the handling characteristics of the bulk drug in production.

Table A

Drying Time Experiments/Results

Lot ID	Drying Method	Time (hrs)	Solvent Content
CD-2969C	Vac. Tray Dryer	48	1.12% EtOH
	@ 72°C	24	1.08% EtOH
	@ 80°C	72	0.92% EtOH; 2.84% H_2O
CD-3032	Vac. Tray Dryer	24	7.0% EtOH
Milled here	@ 82°C	24	6.0% EtOH
	→ @ 82°C	24	5.5% EtOH
	@ 95°C	24	4.4% EtOH
	@ 95°C	72	0.9% EtOH; 1.24% H_2O
CD-3044	Vac. Tray Dryer @ 60°C	24	5.2% EtOH
Nitrogen bleed	@ 82°C	24	4.1% EtOH
started here	@101°C	24	4.1% EtOH
	→ @ 102°C	18	2.0% EtOH
	@101°C	72	0.2% EtOH
	@101°C	72	0.1% EtOH; 1.5% H_2O
CD-3055	Vac. Tray Dryer @ 60°C	24	7.7% EtOH
Milled; moved	→ @ 82°C	24	5.5% EtOH
into a rotary	→ @ 104°C	24	4.7% EtOH
dryer here	@ 105°C	24	3.4% EtOH
	@ 104°C	24	2.9% EtOH
	@ 101°C	24	2.5% EtOH
	@ 104°C	24	2.0% EtOH
	@ 101°C	24	2.0% EtOH
Moved to trays	→ @ 103°C	24	0.3% EtOH
	@ 103°C	24	0.1% EtOH; 1.92% H_2O

Table A (cont'd)

Drying Time Experiments/Results

Lot ID	Drying Method	Time (hrs)	Solvent Content
CD-3082	Vac. Tray Dryer @ 82°C	24	5.97% EtOH
Milled here	→ @ 98°C	24	0.85% EtOH
Added 5 L	→ @ 97°C	24	0.72% EtOH
water in a tray	@ 97°C	24	0.41% EtOH
here	remilled		0.11% EtOH; 1.59% H_2O
CD-3089	Agitated Pan Dryer-G		
Moved to tray	@ 82°C	24	7.1% EtOH
dryer. Added	@ 98°C	24	5.5% EtOH
5 L water	@ 100°C	24	5.1% EtOH
	→ @ 100°C	20	1.8% EtOH
	@101°C	18	0.22% EtOH
	@100°C	24	0.01% EtOH; 3.02% H_2O
CD-3102	Vac. Tray Dryer @ 84°C	20	6.7% EtOH
	@ 95°C	24	3.9% EtOH
	@ 95°C	24	1.77% EtOH
	@ 95°C	24	0.6% EtOH
	@ 95°C	72	ND EtOH; 2.12% H_2O
CD-3111	Vac. Tray Dryer @ 81°C	24	5.4% EtOH
10 trays	@ 98°C	24	0.07% EtOH
without covers.	@ 97°C	24	0.05% EtOH
Trays placed at	milled		ND EtOH; 1.94% H_2O
top of oven			

Table A (cont'd)

Drying Time Experiments/Results

Lot ID	Drying Method	Time (hrs)	Solvent Content
CD-3103*	Agitated Pan Dryer-G		
1 Kg water	@ 80°C	24	5.4% EtOH
added here	→ @ 80°C	24	0.07% EtOH
	@ 100°C	24	0.05% EtOH
	milled		0.06% EtOH; 3.7% H_2O
CD-3130*	Agitated Pan Dryer-G		
2 Kg water	@ 85°C	24	5.9% EtOH
added here	→ @ 80°C	24	0.04% EtOH
	@ 100°C	24	0.06% EtOH
	milled		0.08% EtOH; 4.15% H_2O
CD-3135*	Agitated Pan Dryer-G		
2 Kg water	@ 80°C	24	5.76% EtOH
added here	→ @ 99°C	22	0.02% EtOH
	@ 98°C	5.5	0.02% EtOH
	milled		ND EtOH; 4.38% H_2O
CD-3172*	Agitated Pan Dryer-C		
4 Kg water	@ 80°C	20	7.0% EtOH
added here	→ @ 100-102°C	19	0.2% EtOH
	@ 100°C	24	0.2% EtOH
CD-3321A*	Agitated Pan Dryer-C		
6.4 Kg water	@ 80-85°C	18	6.27% EtOH
added here	→ @ 96-97°C	27	0.23% EtOH
	@ 97-98°C	19	0.06% EtOH

Table A (cont'd)

Drying Time Experiments/Results

Lot ID	Drying Method	Time (hrs)	Solvent Content
CD-3243*	Agitated Pan Dryer-C		
6.4 Kg water	@ 85-87°C	19	7.22% EtOH
added here	→ @ 98-99°C	16	0.09% EtOH
	@ 99°C	18	0.06% EtOH

The product is amorphous by XRD unless designated with * symbol.

The scale of product from CD-3172 was 35.1 Kg; from CD-3221A was 53.9 Kg; from CD-3243 was 49.3 Kg.

* Crystalline product by XRD analysis.

The extra drying time in these examples is because of the 24-hour turn around time for the ethyl alcohol analysis.

Table B

Bulk Density Results

Lot ID	Bulk Density Loose (g/mL)	Bulk Density Tapped (g/mL)	XRD
CD-2969C	0.336	0.439	Amorphous
CD-3032	0.239	0.306	Amorphous
CD-3044	0.249	0.279	Amorphous
CD-3055	0.280	0.315	Amorphous
CD-3082	0.234	0.337	Amorphous
CD-3089	0.292	0.337	Amorphous
CD-3102	0.215	0.270	Amorphous
CD-3111	0.218	0.264	Amorphous
CD-3103	0.343	0.484	Crystalline
CD-3130	0.311	0.496	Crystalline
CD-3135	0.242	0.379	Crystalline
CD-3172	0.281	0.438	Crystalline
CD-3221A	0.372	0.521	Crystalline
CD-3243	0.235	0.300	Crystalline

It is to be understood that while the forms of the present invention described above constitute the best mode contemplated of practicing the present invention, the preceding description is not intended to illustrate all possible forms thereof.

It is further appreciated that one skilled in the art upon reading the description of the present invention will note that various changes may be made to the present invention without departing from the spirit and scope thereof which should be construed according to the appended claims.

All references cited herein are intended to be incorporated by reference to the full extent as if each individual reference was individually and specifically incorporated by reference.

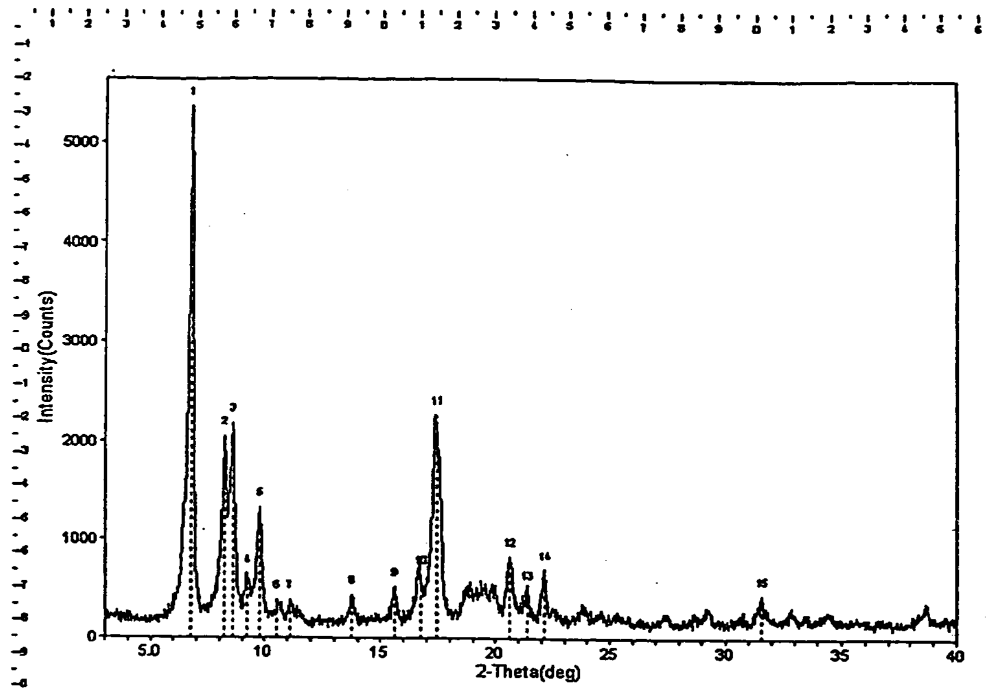


Figure 1

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.760	13.0648	5106	100.0	1497	100.0	0.234
2	8.183	10.7953	1743	34.1	435	29.1	0.200
3	8.560	10.3207	1866	36.5	543	36.3	0.233
4	9.239	9.5638	234	4.6	29	1.9	0.096
5	9.760	9.0546	972	19.0	220	14.7	0.181
6	10.569	8.3634	156	3.1	12	0.8	0.061
7	11.141	7.9353	178	3.5	29	1.9	0.130
8	13.760	6.4304	266	5.2	46	3.1	0.138
9	15.599	5.6761	338	6.6	63	4.2	0.148
10	16.740	5.2917	433	8.5	64	4.3	0.118
11	17.420	5.0866	1890	37.0	689	46.0	0.291
12	20.639	4.3000	523	10.2	128	8.5	0.196
13	21.391	4.1505	188	3.7	20	1.3	0.085
14	22.139	4.0119	445	8.7	74	4.9	0.132
15	31.559	2.8326	270	5.3	24	1.6	0.070

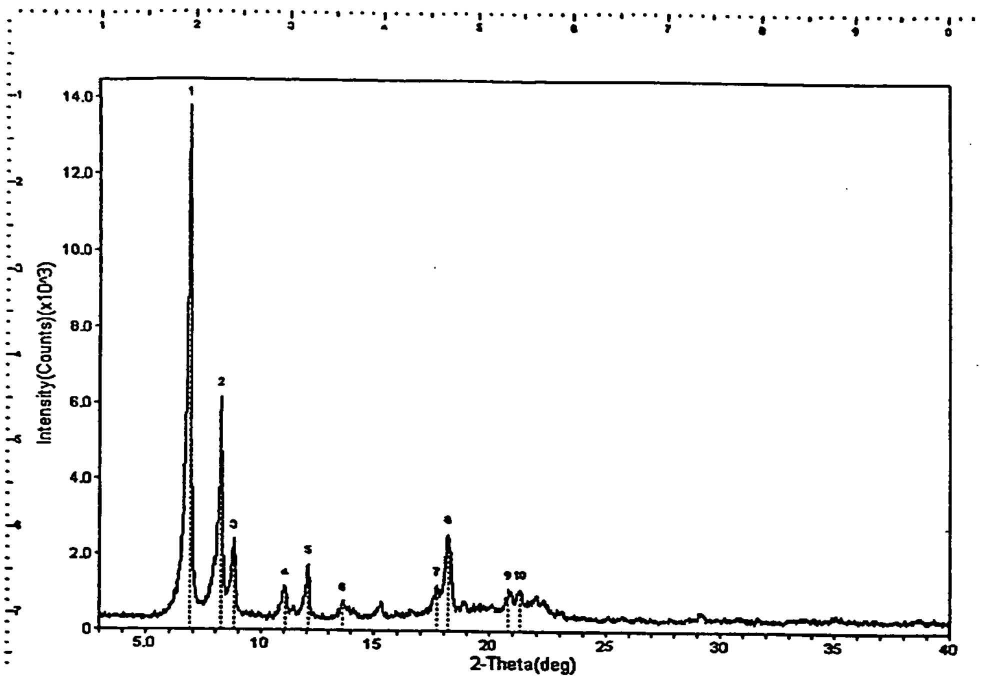


Figure 2

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.899	12.8028	13186	100.0	3025	100.0	0.184
2	8.261	10.6945	5221	39.6	931	30.8	0.143
3	8.838	9.9969	2057	15.6	482	15.9	0.187
4	11.061	7.9927	785	6.0	160	5.3	0.163
5	12.100	7.3086	1355	10.3	150	4.9	0.088
6	13.619	6.4964	450	3.4	89	2.9	0.157
7	17.677	5.0132	753	5.7	126	4.2	0.134
8	18.180	4.8755	2011	15.3	588	19.4	0.234
9	20.840	4.2588	439	3.3	40	1.3	0.072
10	21.334	4.1615	427	3.2	67	2.2	0.125

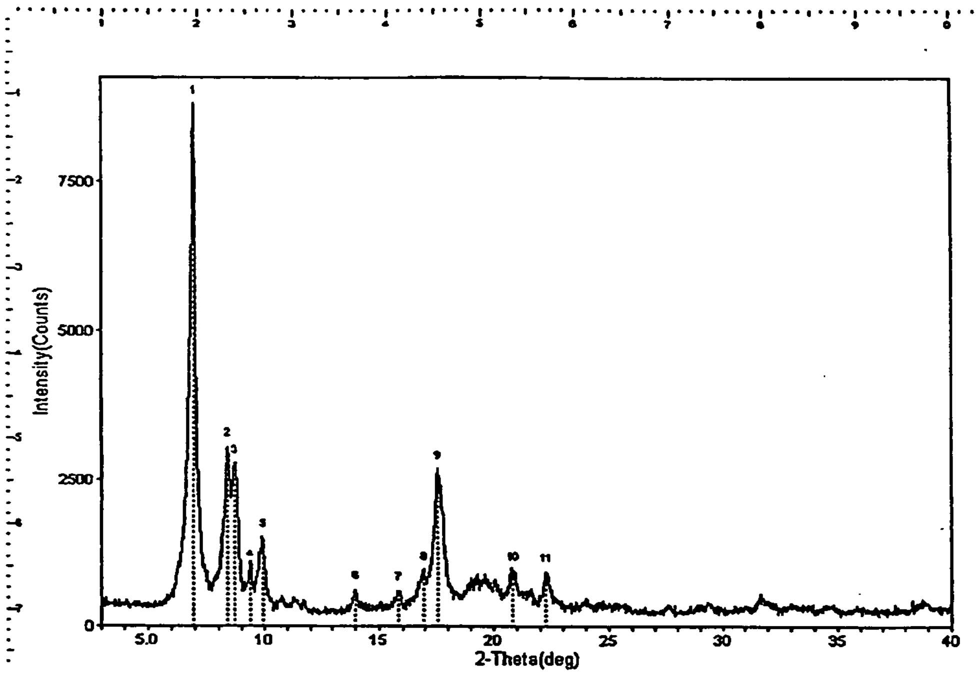


Figure 3

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.959	12.6918	8224	100.0	2809	100.0	0.273
2	8.381	10.5414	2375	28.9	732	26.0	0.246
3	8.701	10.1544	2107	25.6	742	26.4	0.282
4	9.383	9.4176	328	4.0	25	0.9	0.060
5	9.941	8.8906	1160	14.1	356	12.7	0.245
6	13.975	6.3317	330	4.0	26	0.9	0.062
7	15.778	5.6120	244	3.0	38	1.3	0.121
8	16.920	5.2357	597	7.3	213	7.6	0.284
9	17.540	5.0521	2206	26.8	729	25.9	0.264
10	20.799	4.2672	407	4.9	71	2.5	0.138
11	22.261	3.9902	563	6.8	107	3.8	0.152

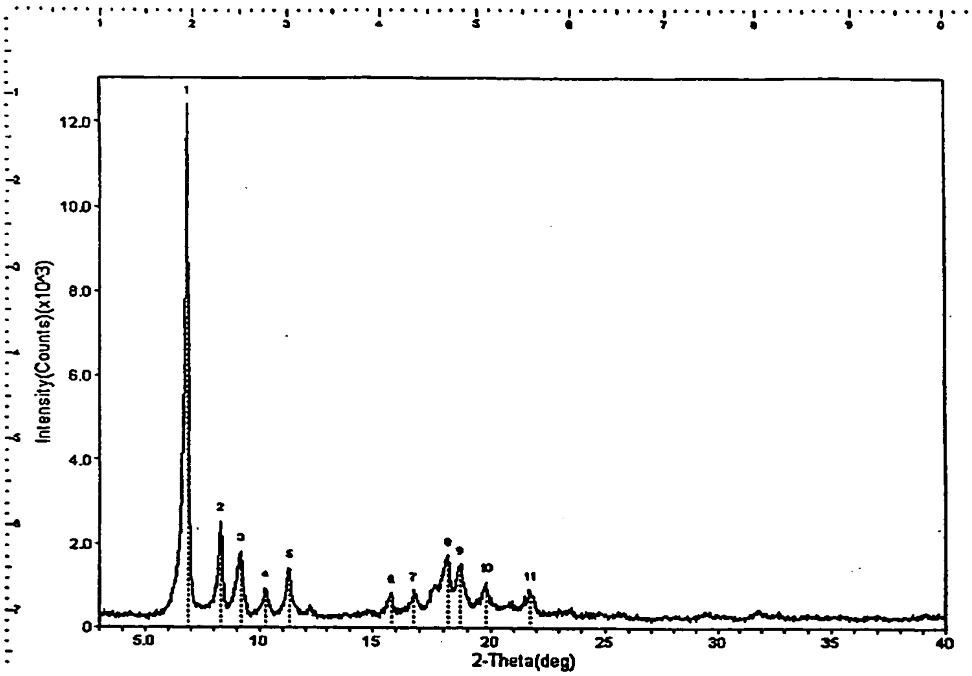


Figure 4

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.896	12.8072	11991	100.0	2593	100.0	0.173
2	8.339	10.5940	2046	17.1	334	12.9	0.131
3	9.219	9.5853	1438	12.0	281	10.8	0.156
4	10.280	8.5979	632	5.3	180	6.9	0.227
5	11.320	7.8105	1079	9.0	322	12.4	0.238
6	15.800	5.6044	463	3.9	59	2.3	0.102
7	16.741	5.2913	432	3.6	38	1.4	0.069
8	18.160	4.8809	1260	10.5	599	23.1	0.380
9	18.702	4.7408	700	5.8	184	7.1	0.210
10	19.816	4.4766	589	4.9	94	3.6	0.127
11	21.724	4.0876	510	4.3	96	3.7	0.150

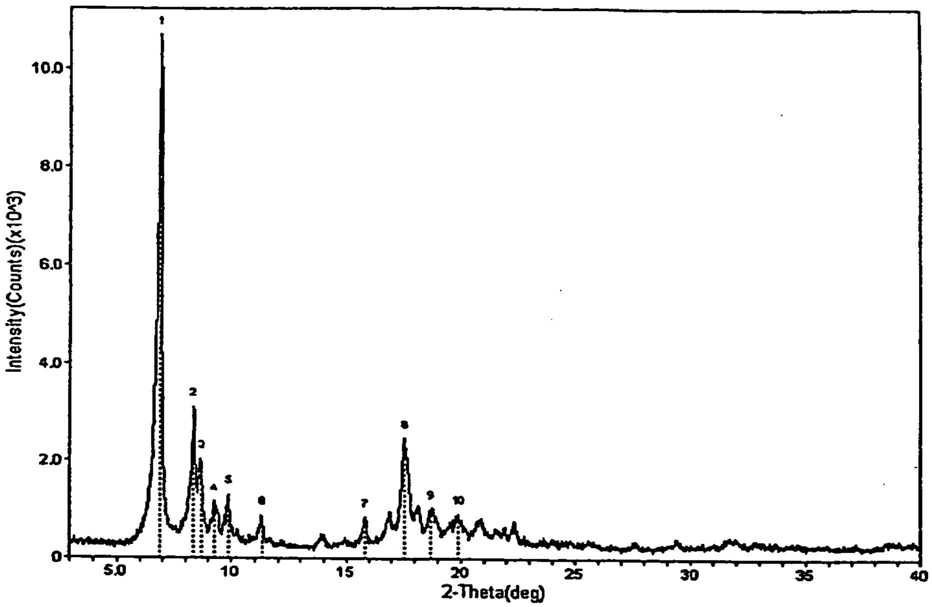


Figure 5

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.901	12.7988	10206	100.0	2683	100.0	0.210
2	8.360	10.5679	2545	24.9	524	19.5	0.164
3	8.680	10.1792	1459	14.3	359	13.4	0.197
4	9.279	9.5230	580	5.7	91	3.4	0.125
5	9.879	8.9456	794	7.8	143	5.3	0.143
6	11.321	7.8094	577	5.7	97	3.6	0.133
7	15.780	5.6113	523	5.1	95	3.5	0.144
8	17.541	5.0519	1710	16.8	418	15.6	0.195
9	18.702	4.7408	459	4.5	116	4.3	0.201
10	19.877	4.4631	403	3.9	67	2.5	0.133

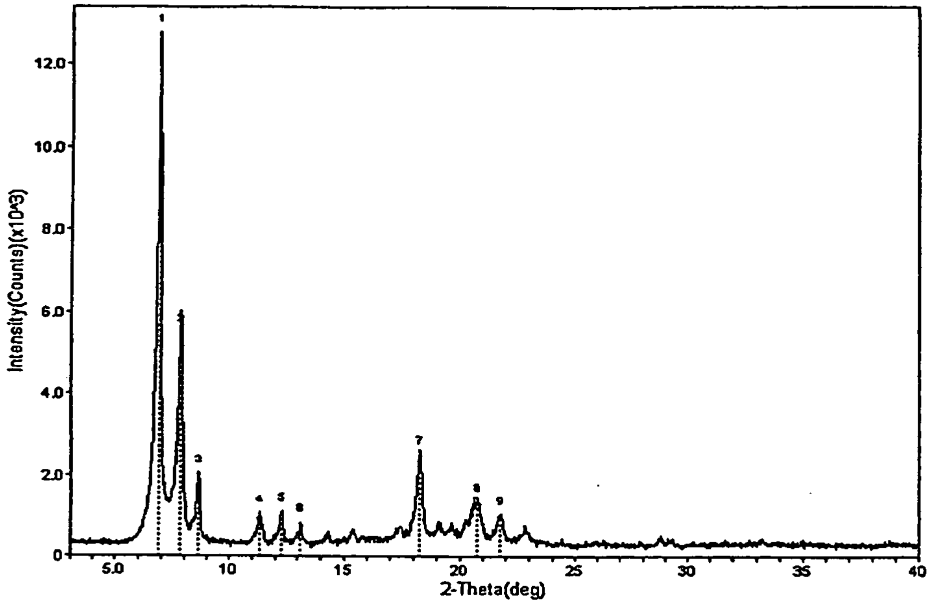


Figure 6

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.899	12.8025	12371	100.0	3495	100.0	0.226
2	7.843	11.2637	4815	38.9	1119	32.0	0.186
3	8.661	10.2009	1709	13.8	357	10.2	0.167
4	11.359	7.7833	771	6.2	141	4.0	0.146
5	12.300	7.1900	752	6.1	127	3.6	0.135
6	13.100	6.7528	517	4.2	37	1.0	0.057
7	18.262	4.8540	1945	15.7	596	17.1	0.245
8	20.721	4.2832	828	6.7	279	8.0	0.269
9	21.740	4.0847	573	4.6	146	4.2	0.203

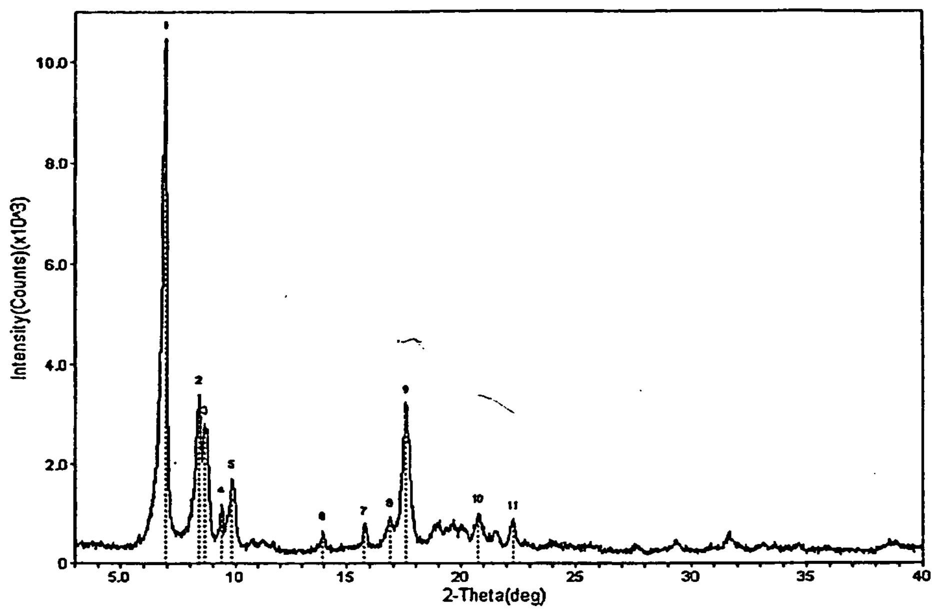


Figure 7

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.939	12.7278	9980	100.0	2717	100.0	0.218
2	8.381	10.5414	2850	28.6	790	29.1	0.222
3	8.640	10.2253	2267	22.7	772	28.4	0.272
4	9.419	9.3815	487	4.9	32	1.2	0.051
5	9.840	8.9812	1288	12.9	255	9.4	0.158
6	13.940	6.3476	374	3.7	56	2.0	0.118
7	15.741	5.6253	450	4.5	45	1.6	0.079
8	16.861	5.2539	580	5.8	192	7.0	0.264
	17.560	5.0464	2604	26.1	846	31.1	0.260
10	20.743	4.2787	508	5.1	73	2.7	0.114
11	22.321	3.9796	542	5.4	156	5.7	0.229

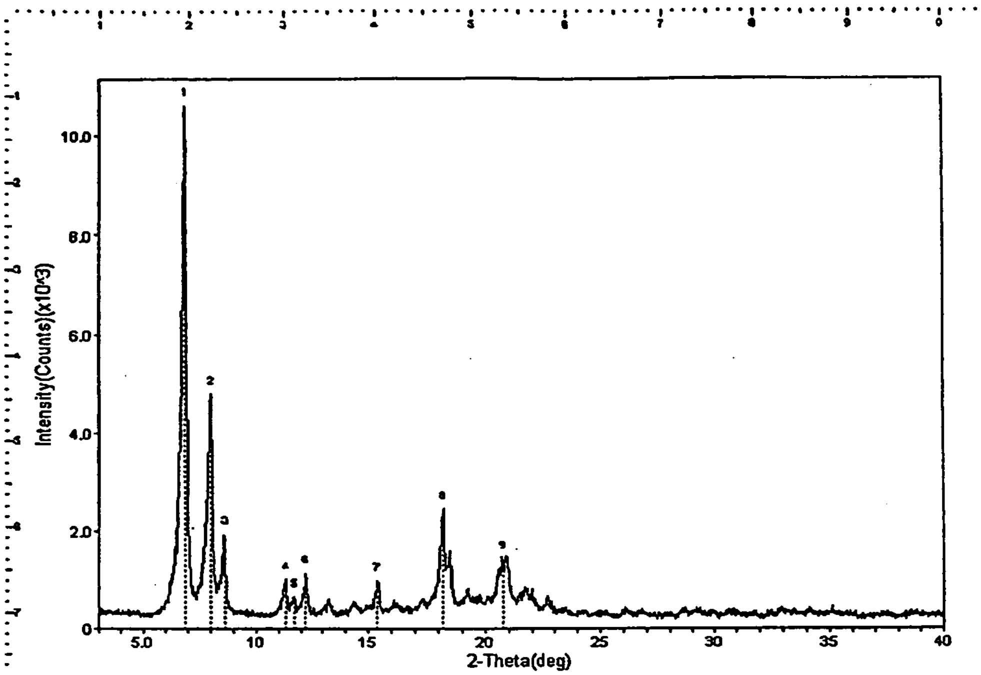


Figure 8

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.918	12.7674	10028	100.0	2562	100.0	0.204
2	8.000	11.0427	3984	39.7	800	31.2	0.161
3	8.619	10.2506	1619	16.1	346	13.5	0.171
4	11.338	7.7981	658	6.6	68	2.6	0.082
5	11.718	7.5459	236	2.4	28	1.1	0.093
6	12.241	7.2243	761	7.6	131	5.1	0.138
7	15.382	5.7557	610	6.1	107	4.2	0.140
8	18.162	4.8803	1937	19.3	441	17.2	0.182
9	20.779	4.2713	853	8.5	222	8.6	0.208

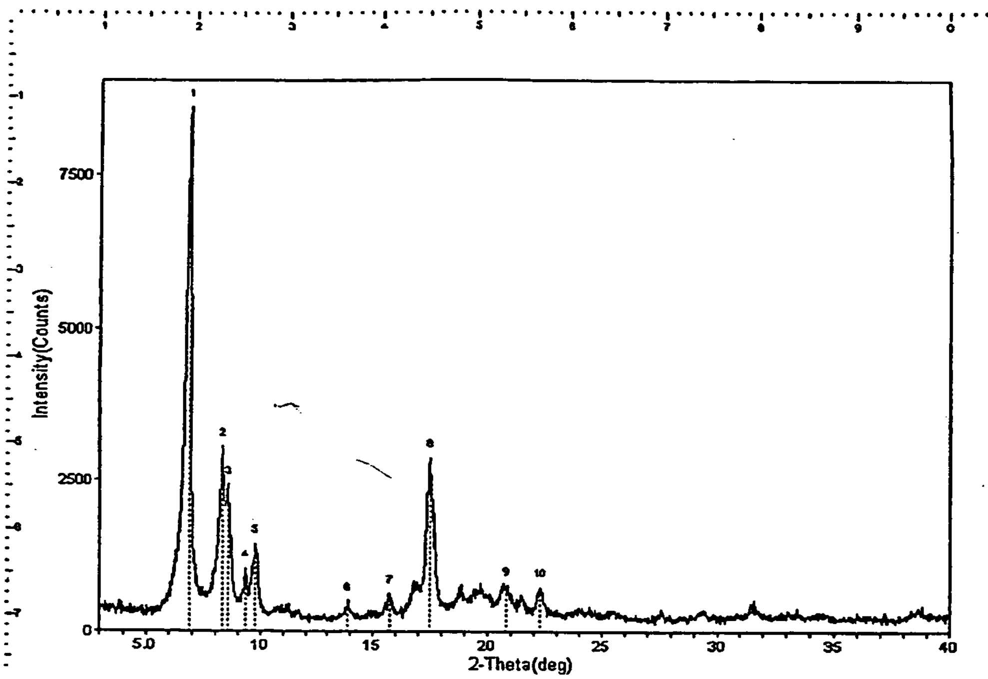


Figure 9

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.877	12.8422	8063	100.0	2195	100.0	0.218
2	8.330	10.6062	2501	31.0	800	36.4	0.256
3	8.581	10.2965	1898	23.5	514	23.4	0.217
4	9.356	9.4446	432	5.4	45	2.0	0.082
5	9.799	9.0191	1064	13.2	275	12.5	0.207
6	13.864	6.3821	293	3.6	58	2.6	0.158
7	15.721	5.6322	312	3.9	67	3.0	0.170
8	17.480	5.0693	2458	30.5	898	40.9	0.292
9	20.818	4.2633	299	3.7	67	3.0	0.178
10	22.280	3.9869	416	5.2	106	4.8	0.202

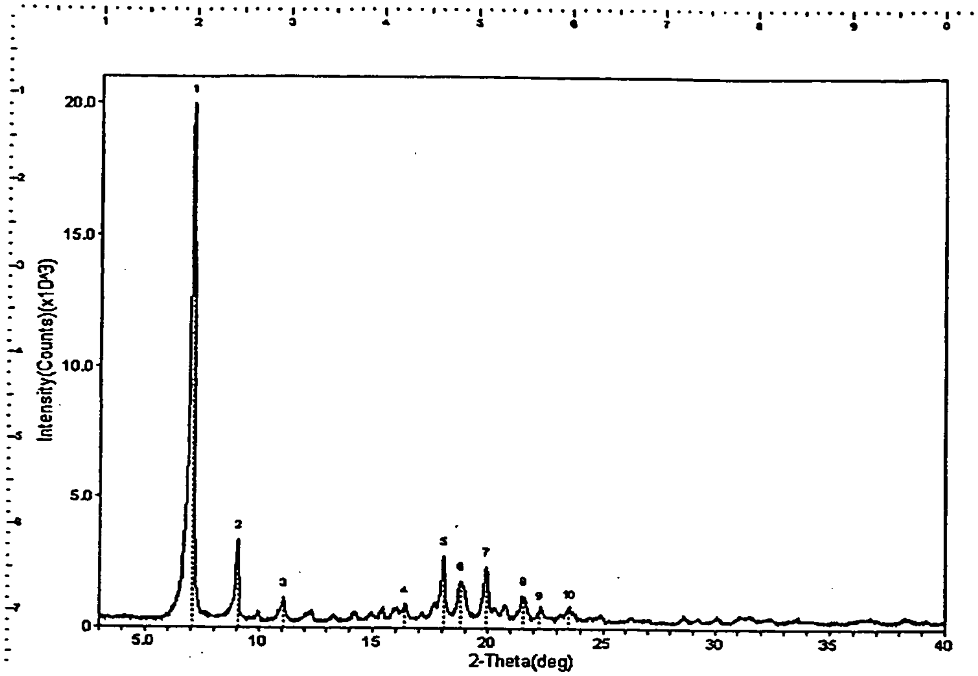


Figure 10

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	7.060	12.5101	19609	100.0	4796	100.0	0.196
2	9.078	9.7332	3027	15.4	567	11.8	0.150
3	11.100	7.9644	924	4.7	164	3.4	0.142
4	16.361	5.4135	554	2.8	76	1.6	0.109
5	18.040	4.9133	2276	11.6	456	9.5	0.160
6	18.820	4.7112	1303	6.6	385	8.0	0.236
7	19.922	4.4532	1886	9.6	457	9.5	0.193
8	21.560	4.1183	853	4.4	205	4.3	0.191
9	22.281	3.9867	343	1.7	37	0.8	0.086
10	23.521	3.7793	450	2.3	107	2.2	0.189

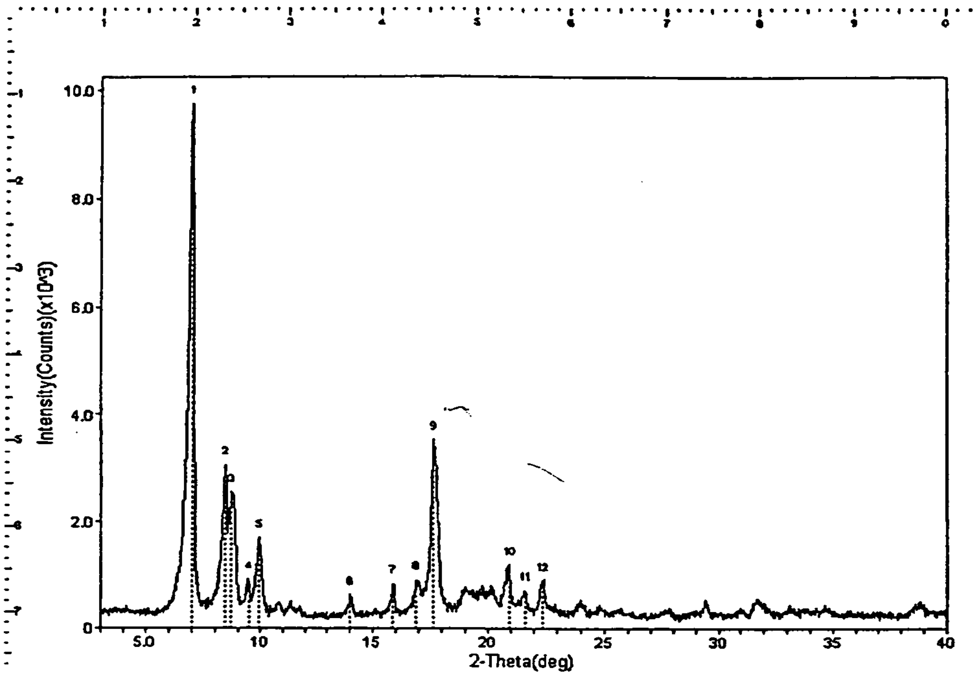


Figure 11

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	7.018	12.5854	9344	100.0	2618	100.0	0.224
2	8.432	10.4775	2599	27.8	676	25.8	0.208
3	8.722	10.1302	2091	22.4	697	26.6	0.266
4	9.499	9.3030	378	4.0	33	1.2	0.069
5	9.980	8.8560	1243	13.3	337	12.9	0.217
6	14.000	6.3206	390	4.2	64	2.4	0.130
7	15.861	5.5830	550	5.9	46	1.7	0.066
8	16.881	5.2479	595	6.4	115	4.4	0.154
9	17.622	5.0287	3006	32.2	1053	40.2	0.280
10	20.918	4.2431	718	7.7	113	4.3	0.126
11	21.641	4.1031	318	3.4	44	1.7	0.110
12	22.380	3.9693	573	6.1	144	5.5	0.201

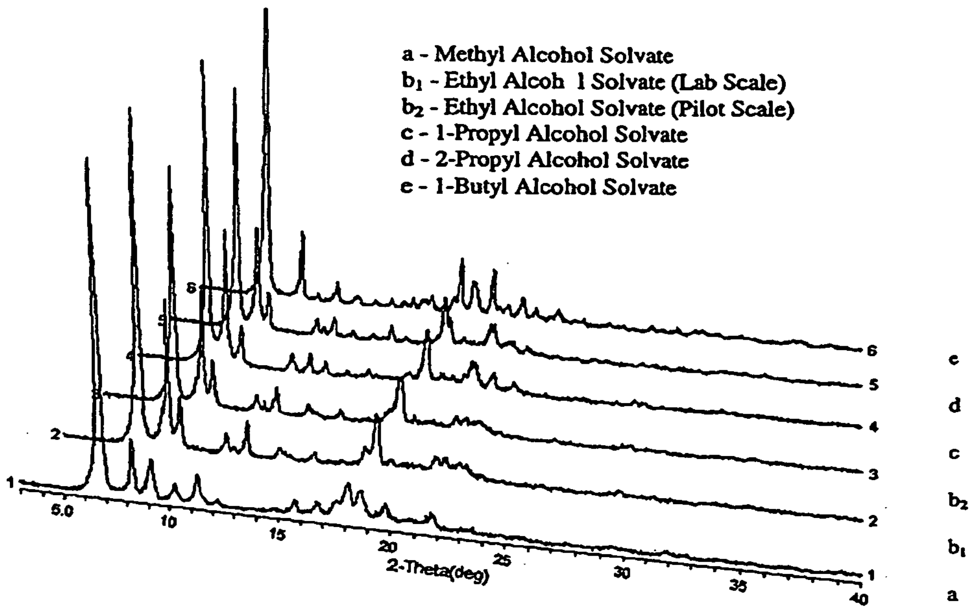


Figure 12. 3-D comparison of X-ray Diffractograms of Alcohol Solvates of 6-(5-Carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic Acid Monocalcium Salt

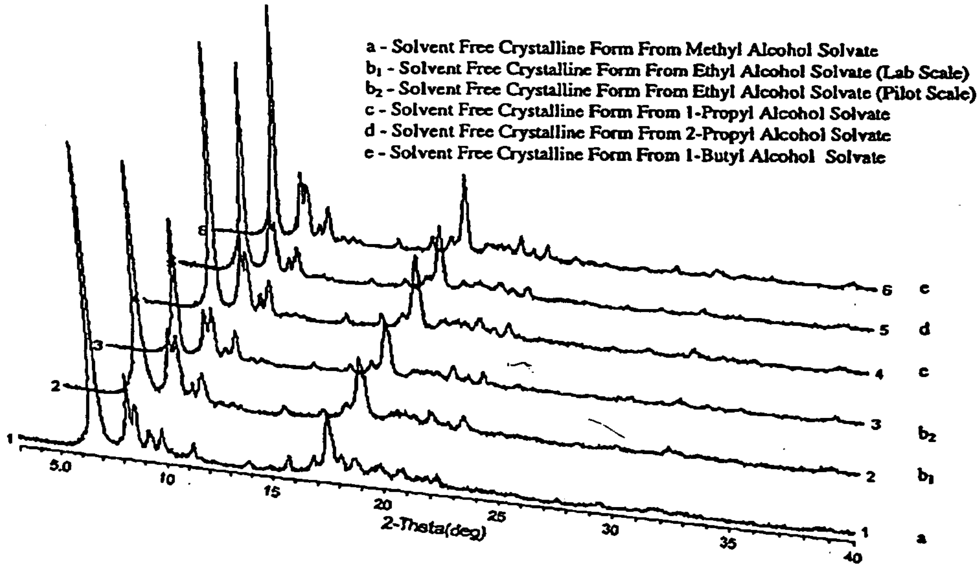


Figure 13. 3-D Comparison of X-ray Diffractograms of Solvent Free 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic Acid Monocalcium Salt (Crystalline Form 1) Produced From Various Alcohol Solvates; CI-1027 (PD 0072953-0038)

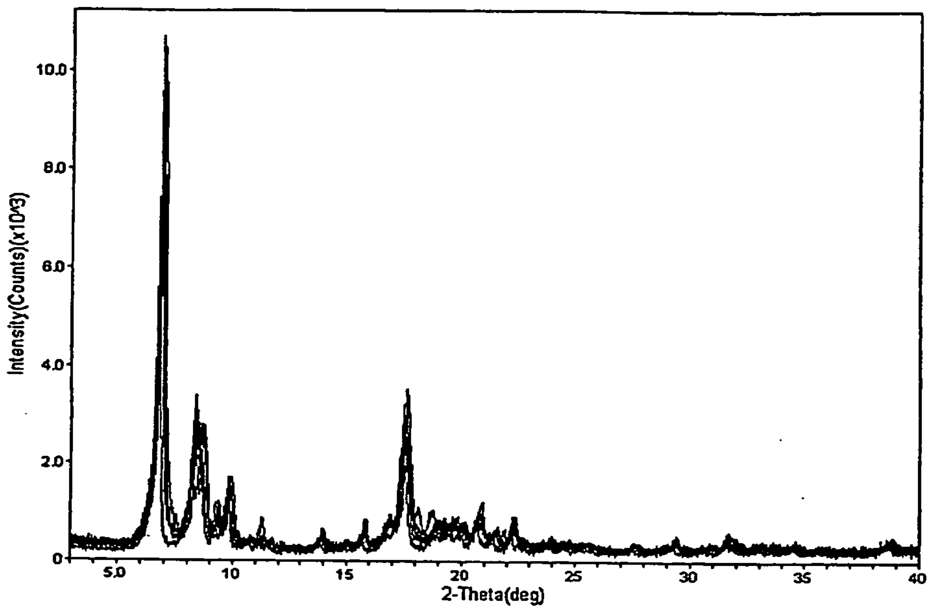


Figure 14 2-D Overlay of X-ray Diffractograms of Solvent Free 6-(5-Carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic Acid Monocalcium Salt (Crystalline Form 1) Produced From Various Alcohol Solvates; CI-1027 (PD 0072953-0038)

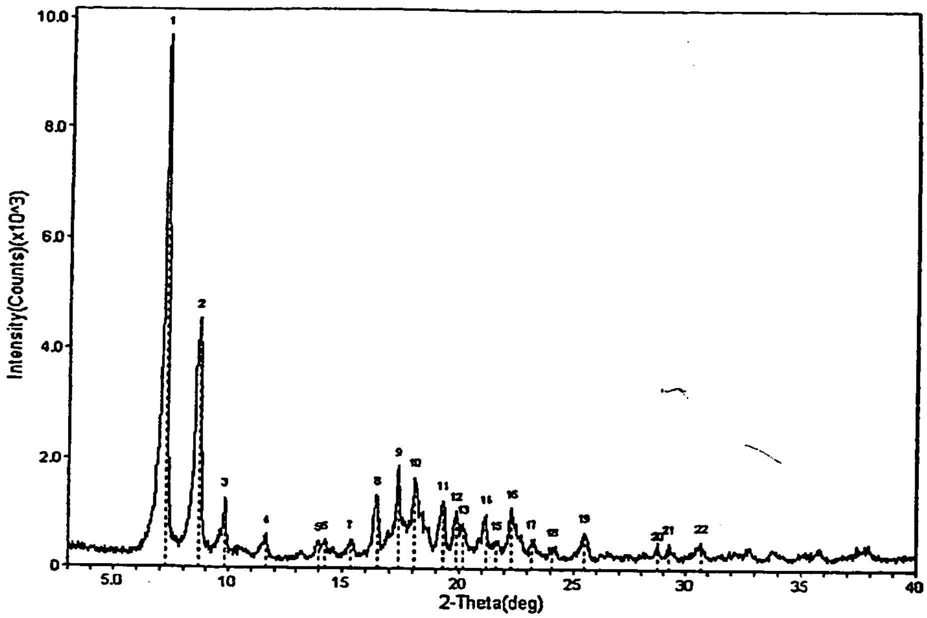


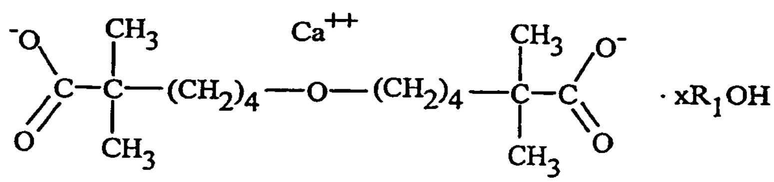
Figure 15

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	7.259	12.1686	9283	100.0	2482	100.0	0.214
2	8.739	10.1100	4191	45.1	603	24.3	0.115
3	93860	8.9628	967	10.4	161	6.5	0.133
4	11.659	7.5838	430	4.6	49	1.9	0.089
5	13.955	6.3408	305	3.3	58	2.3	0.151
6	14.220	6.2233	326	3.5	73	2.9	0.178
7	15.387	5.7537	278	3.0	19	0.7	0.053
8	16.461	5.3806	986	10.6	187	7.5	0.152
9	17.361	5.1039	1490	16.1	348	14.0	0.187
10	18.063	4.9069	1284	13.8	323	13.0	0.201
11	19.302	4.5947	871	9.4	166	6.7	0.152
12	19.862	4.4664	686	7.4	142	5.7	0.166
13	20.200	4.3923	457	4.9	103	4.1	0.179
14	21.178	4.1918	656	7.1	97	3.9	0.117
15	21.641	4.1031	167	1.8	6	0.2	0.029
16	22.300	3.9833	794	8.6	192	7.7	0.193
17	23.218	3.8278	247	2.7	23	0.9	0.071
18	24.100	3.6897	183	2.0	34	1.3	0.145
19	25.481	3.4928	487	5.2	141	5.7	0.231
20	28.800	3.0974	134	1.4	14	0.6	0.083
21	29.297	3.0459	259	2.8	28	1.1	0.084
22	30.700	2.9099	287	3.1	20	0.8	0.055

CLAIMS

What is claimed is:

1. A compound comprising a stable crystalline structure of 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt of the formula:



(II)

CI-1027
PD 0072953-0038
$C_{16}H_{28}O_5Ca \cdot xR_1OH$
$MW = 340.47 \cdot xR_1OH$

wherein R_1 is H or lower alkyl inclusive of methyl, ethyl, propyl, and butyl and $x>0$.

2. The compound of Claim 1, comprising a stable crystalline structure of 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt hydrate.

3. The crystalline compound of Claim 2, wherein said compound is of a first crystalline type having an x-ray powder diffraction pattern comprising:

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.760	13.0648	5106	100.0	1497	100.0	0.234
2	8.183	10.7953	1743	34.1	435	29.1	0.200
3	8.560	10.3207	1866	36.5	543	36.3	0.233
4	9.239	9.5638	234	4.6	29	1.9	0.096
5	9.760	9.0546	972	19.0	220	14.7	0.181
6	10.569	8.3634	156	3.1	12	0.8	0.061
7	11.141	7.9353	178	3.5	29	1.9	0.130
8	13.760	6.4304	266	5.2	46	3.1	0.138
9	15.599	5.6761	338	6.6	63	4.2	0.148
10	16.740	5.2917	433	8.5	64	4.3	0.118
11	17.420	5.0866	1890	37.0	689	46.0	0.291
12	20.639	4.3000	523	10.2	128	8.5	0.196
13	21.391	4.1505	188	3.7	20	1.3	0.085
14	22.139	4.0119	445	8.7	74	4.9	0.132
15	31.559	2.8326	270	5.3	24	1.6	0.070

wherein R_1 is H.

4. The crystalline compound of Claim 3 having a ^{13}C NMR (solid state) in ppm of: 189.6; 186.2; 71.4; 43.4; 30.1; 28.4; 25.2; 23.1.

5. The crystalline compound of Claim 2, wherein said compound comprises 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid mono-calcium salt, wherein R_1 is ethyl.

6. The crystalline compound of Claim 5 having an x-ray powder diffraction pattern comprising:

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.899	12.8028	13186	100.0	3025	100.0	0.184
2	8.261	10.6945	5221	39.6	931	30.8	0.143
3	8.838	9.9969	2057	15.6	482	15.9	0.187
4	11.061	7.9927	785	6.0	160	5.3	0.163
5	12.100	7.3086	1355	10.3	150	4.9	0.088
6	13.619	6.4964	450	3.4	89	2.9	0.157
7	17.677	5.0132	753	5.7	126	4.2	0.134
8	18.180	4.8755	2011	15.3	588	19.4	0.234
9	20.840	4.2588	439	3.3	40	1.3	0.072
10	21.334	4.1615	427	3.2	67	2.2	0.125

7. The crystalline compound of Claim 5 having a ^{13}C NMR (solid state) in ppm of: 189.9; 186.7; 71.6; 58.5; 43.2; 29.9; 23.5.

8. The crystalline compound of Claim 1 wherein said compound comprises 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid mono-calcium salt wherein R_1 is methyl.

9. The crystalline compound of Claim 8, having an x-ray powder diffraction pattern comprising:

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.896	12.8072	11991	100.0	2593	100.0	0.173
2	8.339	10.5940	2046	17.1	334	12.9	0.131
3	9.219	9.5853	1438	12.0	281	10.8	0.156
4	10.280	8.5979	632	5.3	180	6.9	0.227
5	11.320	7.8105	1079	9.0	322	12.4	0.238
6	15.800	5.6044	463	3.9	59	2.3	0.102
7	16.741	5.2913	432	3.6	38	1.4	0.069
8	18.160	4.8809	1260	10.5	599	23.1	0.380
9	18.702	4.7408	700	5.8	184	7.1	0.210
10	19.816	4.4766	589	4.9	94	3.6	0.127
11	21.724	4.0876	510	4.3	96	3.7	0.150

10. The crystalline compound of Claim 8 having a ^{13}C NMR (solid state) in ppm of: 189.6; 186.2; 71.4; 43.2; 29.6; 23.5.

11. The crystalline compound of Claim 1, wherein said compound comprises 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid mono-calcium salt wherein R_1 is 1-propyl.

12. The crystalline compound of Claim 11 having an x-ray powder diffraction pattern comprising:

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.899	12.8025	12371	100.0	3495	100.0	0.226
2	7.843	11.2637	4815	38.9	1119	32.0	0.186
3	8.661	10.2009	1709	13.8	357	10.2	0.167
4	11.359	7.7833	771	6.2	141	4.0	0.146
5	12.300	7.1900	752	6.1	127	3.6	0.135
6	13.100	6.7528	517	4.2	37	1.0	0.057
7	18.262	4.8540	1945	15.7	596	17.1	0.245
8	20.721	4.2832	828	6.7	279	8.0	0.269
9	21.740	4.0847	573	4.6	146	4.2	0.203

13. The crystalline compound of Claim 11 having a ^{13}C NMR (solid state) in ppm of: 189.6; 186.2; 71.4; 43.2; 29.6; 23.5.

14. The crystalline compound of Claim 1, wherein said compound comprises 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid mono-calcium salt wherein R_1 is 2-propyl.

15. The crystalline compound of Claim 14 having an x-ray powder diffraction pattern comprising:

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.918	12.7674	10028	100.0	2562	100.0	0.204

2	8.000	11.0427	3984	39.7	800	31.2	0.161
3	8.619	10.2506	1619	16.1	346	13.5	0.171
4	11.338	7.7981	658	6.6	68	2.6	0.082
5	11.718	7.5459	236	2.4	28	1.1	0.093
6	12.241	7.2243	761	7.6	131	5.1	0.138
7	15.382	5.7557	610	6.1	107	4.2	0.140
8	18.162	4.8803	1937	19.3	441	17.2	0.182
9	20.779	4.2713	853	8.5	222	8.6	0.208

16. The crystalline compound of Claim 14 having a ^{13}C NMR (solid state) in ppm of: 189.4; 187.7; 70.9; 69.4; 66.5; 63.8; 43.2; 35.0; 30.1; 23.8; 18.7; 14.3.

17. The crystalline compound of Claim 1, wherein said compound comprises 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid mono-calcium salt wherein R_1 is 1-butanol.

18. The crystalline compound of Claim 17 having an x-ray powder diffraction pattern comprising:

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	7.060	12.5101	19609	100.0	4796	100.0	0.196
2	9.078	9.7332	3027	15.4	567	11.8	0.150
3	11.100	7.9644	924	4.7	164	3.4	0.142
4	16.361	5.4135	554	2.8	76	1.6	0.109
5	18.040	4.9133	2276	11.6	456	9.5	0.160
6	18.820	4.7112	1303	6.6	385	8.0	0.236
7	19.922	4.4532	1886	9.6	457	9.5	0.193
8	21.560	4.1183	853	4.4	205	4.3	0.191
9	22.281	3.9867	343	1.7	37	0.8	0.086
10	23.521	3.7793	450	2.3	107	2.2	0.189

19. The compound of Claim 17 having a ^{13}C NMR (solid state) in ppm of: 189.9; 186.0; 71.6; 43.2; 29.9; 23.8.

20. The crystalline compound of Claim 1, wherein said compound is a second crystalline type having an x-ray powder diffraction pattern comprising:

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	7.259	12.1686	9283	100.0	2482	100.0	0.214
2	8.739	10.1100	4191	45.1	603	24.3	0.115
3	93860	8.9628	967	10.4	161	6.5	0.133
4	11.659	7.5838	430	4.6	49	1.9	0.089
5	13.955	6.3408	305	3.3	58	2.3	0.151
6	14.220	6.2233	326	3.5	73	2.9	0.178
7	15.387	5.7537	278	3.0	19	0.7	0.053
8	16.461	5.3806	986	10.6	187	7.5	0.152
9	17.361	5.1039	1490	16.1	348	14.0	0.187
10	18.063	4.9069	1284	13.8	323	13.0	0.201
11	19.302	4.5947	871	9.4	166	6.7	0.152
12	19.862	4.4664	686	7.4	142	5.7	0.166
13	20.200	4.3923	457	4.9	103	4.1	0.179
14	21.178	4.1918	656	7.1	97	3.9	0.117
15	21.641	4.1031	167	1.8	6	0.2	0.029
16	22.300	3.9833	794	8.6	192	7.7	0.193
17	23.218	3.8278	247	2.7	23	0.9	0.071
18	24.100	3.6897	183	2.0	34	1.3	0.145
19	25.481	3.4928	487	5.2	141	5.7	0.231
20	28.800	3.0974	134	1.4	14	0.6	0.083
21	29.297	3.0459	259	2.8	28	1.1	0.084
22	30.700	2.9099	287	3.1	20	0.8	0.055

wherein R_1 is H.

21. The crystalline compound of Claim 20 having a ^{13}C NMR (solid state) in ppm of: **[results pending]**.

22. The compound of Claim 1, wherein said crystalline structure contains from approximately 0.1 to approximately 1.0 water molecules per salt ion.

23. A method of synthesizing a stable crystalline dicarboxylate acid ether salt comprising the steps of: providing a compound of the formula

(I)

reacting the compound with calcium oxide in an organic solvent wherein the calcium oxide is allowed sufficient time for a reaction to occur so as to yield a solid product; and drying the solid product to obtain the monocalcium dicarboxylate ether salt of the compound having a stoichiometric ratio of calcium to dicarboxylate form of the compound of approximately 1:1.

24. The method of Claim 23, wherein the organic solvent is a C_1-C_{12} alcohol.

25. The method of Claim 23, wherein the organic solvent is essentially anhydrous.

26. The method of Claim 23 further comprising the step of introducing a work-up solvent to the organic alcohol solvent following the step of allowing sufficient time for the reaction to occur wherein the work-up solvent causes at least a portion of the monocalcium dicarboxylate ether salt to precipitate from the organic alcohol solvent.

27. The method of Claim 26 wherein the work-up solvent is methyl *tert*-butyl ether.

28. The method of Claim 23 further comprising the step of filtering the solid product from the organic solvent prior to drying.

29. The method of Claim 23 further comprising the step of washing the solid product with the organic work-up solvent subsequent to filtering.

30. The method of Claim 23 further comprising the steps of humidifying, agitating, and heating the solid product prior to drying the solid product.

31. The method of Claim 23 wherein the solid product contains between approximately 0.1 and approximately 1.0 equivalents of water per equivalent of the monocalcium dicarboxylate ether salt subsequent to said filtering step and said drying step.

32. The method of Claim 23 wherein said reacting step occurs at a temperature between about 15°C and the reflux point of the organic solvent at standard pressure.

33. The method of Claim 23 wherein said reacting step occurs at a temperature between the reflux point of the organic solvent and about 150°C at a pressure above standard pressure.

34. A method of converting a first crystalline form of the compound 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt to a second crystalline form of the compound 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt, said method comprising the steps of: exposing the compound to water; agitating the compound and water; heating the compound and water for sufficient time for a conversion to occur so as to yield a solid product; and drying the solid product to obtain the second crystalline form of the monocalcium dicarboxylate ether salt of the compound, wherein the second crystalline form has a stoichiometric ratio of calcium to dicarboxylate form of the compound of 1:1.

35. The method of Claim 34 further comprising the step of filtering the second crystalline form solid product from the water prior to said drying step.

36. A vascular disease treatment formulation comprising as an active ingredient a stable crystalline form of a compound of 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt as set forth in Claim 1 together with one or more pharmaceutically acceptable diluents, carriers or excipients.

37. A vascular disease treatment formulation comprising as an active ingredient a stable crystalline form of a compound of 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt as set forth in Claim 2 together with one or more pharmaceutically acceptable diluents, carriers or excipients.

38. A vascular disease treatment formulation comprising as an active ingredient a stable crystalline form of a compound of 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt as set forth in Claim 20 together with one or more pharmaceutically acceptable diluents, carriers or excipients.

39. A compound as set forth in Claim 1 for use as a pharmaceutical agent.

40. The use of a compound as set forth in Claim 1 for the treatment of vascular disease.

41. The use of a compound as set forth in Claim 1 for the treatment of diabetes.

42. A compound according to Claim 1 substantially as described herein in any of the examples.

43. A method of treating a vascular disease in a patient in need thereof, said method comprising administering to the patient a therapeutically effective amount of a stable crystalline form of the compound 6-(5-carboxy-5-methyl-hexyloxy)-2,2-dimethylhexanoic acid monocalcium salt.

44. A method according to Claim 43, wherein the crystalline form of the compound has an x-ray powder diffraction pattern comprising:

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	6.760	13.0648	5106	100.0	1497	100.0	0.234
2	8.183	10.7953	1743	34.1	435	29.1	0.200
3	8.560	10.3207	1866	36.5	543	36.3	0.233
4	9.239	9.5638	234	4.6	29	1.9	0.096
5	9.760	9.0546	972	19.0	220	14.7	0.181
6	10.569	8.3634	156	3.1	12	0.8	0.061
7	11.141	7.9353	178	3.5	29	1.9	0.130
8	13.760	6.4304	266	5.2	46	3.1	0.138
9	15.599	5.6761	338	6.6	63	4.2	0.148
10	16.740	5.2917	433	8.5	64	4.3	0.118
11	17.420	5.0866	1890	37.0	689	46.0	0.291
12	20.639	4.3000	523	10.2	128	8.5	0.196
13	21.391	4.1505	188	3.7	20	1.3	0.085
14	22.139	4.0119	445	8.7	74	4.9	0.132
15	31.559	2.8326	270	5.3	24	1.6	0.070

45. A method according to Claim 43, wherein the crystalline form of the compound has an x-ray powder diffraction pattern comprising:

#	2-Theta	d(A)	Peak	P%	Area	Area%	FWHM
1	7.259	12.1686	9283	100.0	2482	100.0	0.214
2	8.739	10.1100	4191	45.1	603	24.3	0.115
3	.93860	8.9628	967	10.4	161	6.5	0.133
4	11.659	7.5838	430	4.6	49	1.9	0.089
5	13.955	6.3408	305	3.3	58	2.3	0.151
6	14.220	6.2233	326	3.5	73	2.9	0.178
7	15.387	5.7537	278	3.0	19	0.7	0.053
8	16.461	5.3806	986	10.6	187	7.5	0.152
9	17.361	5.1039	1490	16.1	348	14.0	0.187
10	18.063	4.9069	1284	13.8	323	13.0	0.201
11	19.302	4.5947	871	9.4	166	6.7	0.152
12	19.862	4.4664	686	7.4	142	5.7	0.166
13	20.200	4.3923	457	4.9	103	4.1	0.179
14	21.178	4.1918	656	7.1	97	3.9	0.117
15	21.641	4.1031	167	1.8	6	0.2	0.029
16	22.300	3.9833	794	8.6	192	7.7	0.193
17	23.218	3.8278	247	2.7	23	0.9	0.071
18	24.100	3.6897	183	2.0	34	1.3	0.145
19	25.481	3.4928	487	5.2	141	5.7	0.231
20	28.800	3.0974	134	1.4	14	0.6	0.083
21	29.297	3.0459	259	2.8	28	1.1	0.084
22	30.700	2.9099	287	3.1	20	0.8	0.055

ABSTRACT

Calcium dicarboxylate ether salts are crystalline compounds amenable to the treatment of vascular diseases and noninsulin dependent diabetes mellitus. Methodology for the production of stable crystalline monocalcium dicarboxylate ether salts provides a convenient source for these novel compounds. Two stable crystalline forms of monocalcium dicarboxylate ether salts are disclosed.